A COMPARISON OF THE UTILIZATION OF NUCLEIC ACIDS OF ANIMAL AND VEGETABLE ORIGIN

by

T. BRAILSFORD ROBERTSON, C. STANTON HICKS, AND HEDLEY R. MARSTON

(From the Department of Biochemistry and General Physiology and the Department of Human Physiology and Pharmacology of the University of Adelaide).

(Submitted for publication 5th August, 1927.)

OBJECTS OF THE EXPERIMENTS.

For some years past investigations have been proceeding in the Department of Biochemistry and General Physiology with the object of ascertaining the effects of administration of nucleic acids of animal and vegetable origins upon the growth and life-history of animals. One result of these experiments, which will be described in a subsequent communication, has been the discovery that certain modifications in the life-history of the animals which are induced by the administration of nucleic acid differ in quantity, if not in kind, with the source, vegetable or animal, of the nucleic acid. This fact suggested the possibility that nucleic acids of animal and vegetable origin may be utilized by animals in different ways. If both forms of nucleic acid were broken down into their constituent phosphoric acid, carbohydrate, and nitrogenous moieties in the alimentary canal, then, it is true, one would anticipate little, if any, difference in their effects upon the animal, for the pentose yielded by vegetable nucleic acid would be substantially equivalent to the hexose yielded by animal nucleic acid, so far as utilization in metabolism is concerned, and otherwise the products of decomposition of the two nucleic acids would differ only in the substitution, among the products derived from animal nucleic acid, of methyl-uracil (thymine) for the uracil which is present in nucleic acid of vegetable origin (13).

So far as the final products of decomposition are concerned, therefore, the two types of nucleic acid might be expected to be virtually equivalent to one another. According to Abderhalden (1), however, the breakdown of nucleic acid in the alimentary canal ceases with the formation of nucleotides and nucleosides, the latter being absorbed directly into the bloom-stream (5), where they are

thereafter demonstrable (20). If this be the case, and if nucleic acid were utilizable, wholly or in part, in the form of nucleotides, or even as nucleosides, then in so far as this type of utilization is concerned the two substances would no longer be equivalent, for nucleotides of vegetable origin cannot be converted into nucleotides of the animal type without preliminary decomposition, to permit the insertion, in the middle of the molecule, of a hexose molecule in place of a pentose. Thus if any appreciable proportion of nucleic acid can be utilized in an undecomposed or partially decomposed form, nucleic acids of animal and vegetable origins should differ to that extent in their utilizability by the animal.

There are not wanting evidences of an inconclusive but suggestive character that such a difference of utilizability may actually subsist between nucleic acids of animal and vegetable origin. Thus Jones (12) has shown that the pancreas of the pig contains an enzyme capable of decomposing plant nucleic acid into its nucleotides, while it does not split animal nucleic acid. Levene and Medigreceanu (15) have found that whereas both types of nucleic acid are split by enzymes contained in the digestive juices, yet animal nucleic acid is split very much more slowly than nucleic acid of vegetable origin. Ringer and Underhill (19) have shown that vegetable nucleic acid when administered intravenously elicits symptoms of shock, with evident upset of nitrogenous metabolism, while animal nucleic acid, similarly administered, does not elicit any such response. In other words, vegetable nucleic acid, as such, behaves in the circulation like a foreign substance, while animal nucleic acid is tolerated without any appreciable disturbance of metabolism.

Numerous studies have been made of the effects of administering nucleic acid by mouth to human beings. These studies have been summarized by Givens and Hunter (10), but it is significant that in their summary no distinction is drawn by these authors between the two types of nucleic acid, although some of the authors whom they cite employed the one type of nucleic acid and some the other. The majority of investigators have confined themselves to ascertaining the effect of nucleic acid administration upon the uric acid output of the subjects. A few have extended the scope of their analyses to include the total nitrogen and urea output. There does not appear heretofore to have been any attempt to ascertain the effect of the administration of nucleic acid upon the output of other important metabolites or upon the properties of the urine. The doses administered have usually been comparatively small; the largest amount administered in any of the experiments quoted by Hunter and Givens having been a total of 50 grms. of a very impure preparation (as its purine content shows), administered by Frank and Schittenhelm (9) throughout the duration of an experiment lasting five days. Finally, no one, so far as we are aware, has ever attempted to compare the effects of administration of the two types of nucleic acid by mouth *to the same subjects*. Only a comparison of this kind, obviously, can throw any light upon the relative utilizability of the two types.

With the object of obtaining further information concerning the possibility outlined above, we have sought to ascertain the comparative effects upon the same subjects of oral administration of animal and vegetable nucleic acids in massive doses, superadded to a purine-free diet.

METHODS.

Two experiments were carried out, each of them upon two subjects, C.S.H. and T.B.R. in the one case, and C.S.H. and H.R.M. in the other. The diet consisted of white bread, milk, butter, cheese, eggs, potatoes, and, with the subjects C.S.H. and H.R.M., a little fruit (apple or prunes). Water, wine, and whisky were drunk, but no tea, coffee, cocoa, or beer. In the first experiment, with subjects C.S.H. and T.B.R. no stated quantities were eaten, but appetite in the case of one of us (T.B.R.) was practically uniform throughout the twenty days' duration of the experiment. This was indicated, not only by the amounts eaten, but by the steadiness of the nitrogenous output during the periods when no nucleic acid was being eaten (see charts in figure 1). The other subject (C.S.H.) was somewhat affected by very warm weather which prevailed during the preliminary four-day period of purine-free diet, and as the temperature became more moderate his appetite increased. Nevertheless, a practically uniform protein intake was maintained, an average of four and a half eggs being ingested per diem. The fluctuations of nitrogenous output, which was variable in this subject even during the purine-free period (see charts in figure 2), are to be attributed in the main, therefore, to the temporary condition of the subject, who, as we shall see, suffered a loss of nitrogen throughout the duration of this experiment.

In the second experiment the source of uncertainty constituted by an undefined input of nitrogen was eliminated. Subject C.S.H. varied his foodstuffs slightly, but weighed them daily, and from the weights the nitrogenous input was computed from the standard analyses, tabulated by Lusk (16). Subject H.R.M. ate precisely the same diet daily, after the first twenty-four hours, and the nitrogenous input was therefore constant. The diet of H.R.M. is given below, and so far as constituents and not quantities are concerned it may serve as a description of the diet eaten by the other subjects as well.

DIET CONSUMED BY H.R.M.

First 24 hours.

		Protein.	Fat.	Carbohydrate.	Calories.
Milk	1 litre.	32	38	50	680
Bread	200 grms.	15·1	2·4	105	416
Butter	60 grms.	·6	48·5	—	450
Flour	100 grms.	8·3	1·0	73·5	254

		Protein.	Fat.	Carbohydrate.	Calories.
Eggs	190 grms.	24·7	19·0	—	270
Rice	100 grms.	2·3	—	77	352
Cheese	60 grms.	12·5	16	12	207
Prunes (Dried) ..	50 grms.	·8	—	33	135
Orange Juice	100 cc.	·6	—	10	46
Estimated Intake ..		96·9	124·9	360·5	2,810
Whisky, 250 cc.					
	Subsequent Days.				
Milk	1 litre.	32	38	50	680
Bread	380 grms.	26	4·5	198	790
Butter	60 grms.	·6	48·5	—	450
Eggs	190 grms.	24·7	19·0	—	270
Rice	50 grms.	1·0	—	39	177
Cheese	50 grms.	12·5	16·0	12	207
Prunes or Dried Apricots	50 grms.	·8	—	33	135
	50 grms.	1·8	—	20	125
Orange Juice	100 cc.	·6	—	10	46
Estimated Intake ..		100·0	126·0	362	2,880
Light Wine, 500 cc.					

In each case the entire period of twenty days was divided into five four-day periods. During the first of these nothing was eaten beyond the purine-free diet described above. During the second period the same diet was eaten, with the addition of 15 grms. per day of animal nucleic acid (C.S.H.), or vegetable nucleic acid (T.B.R. and H.R.M.), divided into three doses of 5 grms. each, which were eaten, mixed with a little water, in the morning, at midday, and in the evening respectively. During the third period the diet again consisted of nothing beyond the purine-free materials which have been enumerated. During the fourth period each subject again partook of 15 grms. of nucleic acid per day, but C.S.H. now ate vegetable nucleic acid, while T.B.R. and H.R.M. ate nucleic acid of animal origin. The diet during the fifth and final period against consisted exclusively of purine-free materials. In the case of subject H.R.M. the fifth period was extended to five days.

The vegetable nucleic acid employed in these experiments was derived from yeast and purchased from the British Drug Houses Ltd. Two preparations were used, the one containing 15·70% of nitrogen (theoretical = 16·1%), and 9·17% of phosphorus (theoretical = 9·5%), the other 15·55% of nitrogen and 9·00% of phosphorus. The animal nucleic acid was prepared in this laboratory for the

[1]The manufacture of the large quantities of animal nucleic acid consumed in this experiment, and others which will form the subject of future communications, was rendered possible by a grant from the Cancer Research Fund of the Commonwealth Department of Health, to whom our thanks and acknowledgments are due.

purposes of this and other experiments by Mr. F. Draper.[1] The source of the nucleic acid was ox-spleen, thymus being not procurable in sufficient amounts. The spleen nucleic acid was prepared by a modification of the method of Levene (14), as follows:

The spleens, in 10-lb. lots, were stripped of their connective tissue covering, ground in a mincer, and transferred to 5 litres of water containing 250 grms. of sodium-hydroxide. The mixture was heated to boiling in enamel buckets, being mechanically stirred meanwhile. Boiling was continued for thirty-five minutes. The mixture was then neutralized with glacial acetic acid, the neutral point, after a little experience, being recognizable by the change of colour of the mixture. Fifty cc. of colloidal iron solution (British Drug Houses Ltd.) were then added, and the mixture filtered. A small amount of $CaCl_2$ solution was added to the filtrate to remove excess of colloidal iron,[2] and the solution again filtered after allowing to stand overnight. To 3 litres of this filtrate were added 500 cc. of concentrated HCl and 8 litres of 98% ethyl alcohol, containing 5% of methyl alcohol. The precipitate was collected and washed with ethyl-methyl alcohol mixture until free from HCl, the final washing being with pure ethyl alcohol. The precipitate was dried at 37°C., and used without further purification. It contained 13·97% of nitrogen (theoretical 14·79%) and 9·23% of phosphorus (theoretical 8·73%).

The urines, collected between 8 a.m. and the same hour on the day following, were preservatized with a solution of thymol in toluene. Total nitrogen was determined by the micro-Kjeldahl method of Folin, urea by the urease method, creatinine colourimetrically with picric acid, phosphates by titration with uranium-acetate (subject T.B.R.) or by the colourimetric method of Briggs (subjects C.S.H. and H.R.M.), chlorides by Volhard's method, ammonia by formol titration, and uric acid by the following modification of the method of Morris and McLeod (17), which has been employed in this laboratory since shortly after the appearance of Morris and McLeod's article.

The reagents required are the following: *Arseno-18-tungstic acid solution.* A mixture of 100 grms. of hydrated sodium tungstate ($Na_2WO_42H_2O$), 135 grms. of arsenic acid anhydride (AS_2O_5), and 650 cc. of water is boiled over a small flame for four hours. The reagent is then treated with a sufficient amount of bromine water to render the colour a clear yellow or yellowish brown. After boiling off any excess of bromine, distilled water is added to make the volume of the cooled mixture 1 litre. This reagent yields no colour with phenols.

Standard Uric Acid Solution. Prepared by the method of Benedict and Franke (4) to contain 20 milligrammes of uric acid (Pfansteil) per litre.

Sodium Cyanide Solution. A 3·33% solution of sodium cyanide, to which has been added 1 drop of strong ammonia per litre.

---

[2]Otherwise the colloidal iron is carried down by the nucleic acid, and the precipitate is in consequence of a very dark colour and heavily contaminated with iron.

Dilute Hydrochloric Acid. One volume of concentrated acid mixed with nine volumes of water.

The analysis is carried out as follows:

The urine is thoroughly mixed and 1 cc. is withdrawn from the swirling liquid by means of an Ostwald pipette,[3] and introduced into a 100 cc. graduate. Distilled water is added to the 20 cc. mark. One cc. of dilute hydrochloric acid is added to balance the acidity of the uric acid standard.[4] Ten cc. of sodium cyanide are added, followed by 2 cc. of the arseno-tungstic acid reagent. The contents of the graduate are mixed, and at the end of five minutes are diluted to the 100 cc. mark and again mixed. The blue solution is compared in a colourimeter with a simultaneously prepared solution, obtained by treating 20 cc. of the standard uric acid solution (containing 0·4 mg. of uric acid) in a 100 cc. graduate, with 10 cc. of the sodium cyanide solution and 2 cc. of the arseno-tungstic acid reagent, diluting the mixture to the 100 cc. mark at the end of five minutes.

The faeces which were passed in each day were evaporated nearly to dryness *in vacuo* over a boiling water bath, drying being completed by heating the residue in an open dish on the boiling water bath. The residues were weighed and then coarsely pulverized. Five gramme samples from each day's passage were ignited for several hours, ignition being aided in the cases of subjects T.B.R. and H.R.M. by addition of a few drops of strong nitric acid. The ash was almost completely soluble in 5 cc. of hot concentrated hydrochloric acid. The very small residue was re-extracted with a few drops of hot concentrated hydrochloric acid. The solution, washing and residue of silicates, were introduced into a 100 cc. flask, and when cool diluted to the 100 cc. mark. The phosphoric acid was determined in 1 cc. of this solution by the colourimetric method of Briggs, using for comparison a standard containing either 0·5 or 1·0 mg. of phosphorus.

In the second experiment upon subject C.S.H. the concentration of uric acid in the blood was determined on the last day of each of the four-day periods. Five cc. of blood was withdrawn from the median basilic vein and deproteinized by the method of Folin. The uric acid in the protein-free filtrates was determined by the method of Benedict (2).[5]

---

[3]In many of the urines passed during the periods of vegetable nucleic acid ingestion, the crystals of uric acid were so large and numerous as to render very questionable the accuracy of sampling by this method. In such cases one of two procedures was adopted. Either a measured quantity of normal NaOH was added to the whole of the urine until the greater part of the uric acid was dissolved, allowance being subsequently made for the alteration of volume and titratable acidity thus brought about, or else a large aliquot was subtracted from the whole volume, and to this 10% NaOH was added until the uric acid was dissolved, allowance being made in the analysis for the dilution of the urine by the sodium hydroxide which was added.

[4]This is essential because the colour which subsequently develops varies with the reaction of the mixture.

[5]We desire to express our indebtedness to Mr. F. Draper for his kindness in carrying out these blood analyses for us.

TABLE 1. SUBJECT T.B.R.

Date	*Urine.* Volume cc.	Titratable Acidity in cc. tenth normal alkali per day	Ammonia Grammes	Urea + Ammonia Nitrogen Grammes	Total Nitrogen Grammes	Creatinine Grammes	Uric Acid Grammes	P_2O_5 Grammes	Chlorides as NaCl Grammes	*Faeces.* Total Weight	P_2O_5 per cent.	Total P_2O_5 Grammes
Purine-free Period.												
29.XI.26	790	395	0·56	11·06	12·85	1·81	0·41	2·05	Not determined	78·6	3·87	3·04
30.XI.26	700	434	0·57	10·50	12·61	2·08	0·38	2·19	Not determined	None	—	—
1.XII.26	745	462	0·60	10·88	13·80	2·12	0·36	2·28	Not determined	23·9	5·63	1·35
2.XII.26	820	435	0·63	11·63	13·33	2·02	0·40	2·57	Not determined	None	—	—
Vegetable Nucleic Acid Period.												
3.XII.26	1,230	689	0·84	14·39	15·97	2·32	0·91	3·75	Not determined	19·94	8·43	1·68
4.XII.26	1,230	738	0·98	12·94	15·50	2·20	1·05	3·91	Not determined	None	—	—
5.XII.26	1,510	695	1·10	12·74	14·66	2·39	1·10	3·96	11·3	43·5	9·89	4·31
6.XII.26	1,870	767	1·59	13·31	15·20	2·43	1·31	4·59	13·9	None	—	—
Purine-free Period.												
7.XII.26	920	644	1·19	10·95	13·05	2·26	0·50	3·56	9·5	41·4	7·19	2·97
8.XII.26	1,060	551	0·92	11·78	12·93	2·14	0·49	2·80	14·2	10·3	4·98	0·51
9.XII.26	880	484	0·81	10·30	11·73	2·09	0·35	2·45	11·3	None	—	—
10.XII.26	700	476	0·73	9·93	11·38	2·02	0·29	2·29	7·0	8·5	5·26	0·45
Animal Nucleic Acid Period.												
11.XII.26	1,090	600	0·93	13·30	14·63	2·43	0·50	3·17	9·1	85·8	5·78	4·96
12.XII.26	970	601	1·06	12·05	13·75	2·32	0·49	3·42	8·7	None	—	—
13.XII.26	1,470	617	1·10	12·86	14·77	2·21	0·56	3·50	16·5	19·18	9·71	1·86
14.XII.26	1,200	660	1.16	13·04	14·63	2·18	0·61	3·72	11·3	None	—	—
Purine-free Period.												
15.XII.26	1,020	551	1·02	11·79	13·86	2·26	0·49	3·02	13·3	None	—	—
16.XII.26	790	490	0·87	11·12	12·25	2·18	0·49	2·45	10·1	36·2	8·94	3·23
17.XII.26	630	447	0·80	11·15	12·11	1·96	0·37	2·19	6·8	11·1	8·43	0·94
18.XII.26	780	460	0·72	12·68	14·31	2·14	0·46	2·49	8·7	None	—	—

EFFECTS OF THE ADMINISTRATION OF NUCLEIC ACIDS UPON EXCRETION.

Considering in the first place the results obtained with subject T.B.R., it is evident from the charts (figure 1) that the volume of urine and its titratable acidity, ammonia, phosphate, uric acid, urea, and total nitrogen contents were all increased during the experimental periods, but very much more during the vegetable nucleic acid period than during the period when animal nucleic acid was partaken. The chlorides merely follow the urinary volume. The discrepancy between the two periods is particularly marked in the case of the uric acid output, which rose to 0·65, 0·70, and 0·91 grms. above the endogenous output during the last three days of the vegetable nucleic acid period, and only attained 0·21 grms. in excess of the endogenous output in the last day of the animal nucleic acid period. The increase of urinary phosphate output during the vegetable nucleic acid period was similarly nearly double the increase which was observed during the period of partaking animal nucleic acid (Table 2).

TABLE 2. SUBJECT T.B.R.

Excess Excretion following Ingestion of Nucleic Acid.

Day.	Vegetable Nucleic Acid. Nitrogen.	P_2O_5. Urine.	P_2O_5. Faeces.	Uric Acid.	Animal Nucleic Acid. Nitrogen.	P_2O_5. Urine.	P_2O_5. Faeces.	Uric Acid.
1st day of Ingestion ..	3·12	1·25	0·68	0·51	1·78	0·67	0·67	0·10
2nd „ „	2·65	1·41	—	0·65	0·90	0·92	—	0·09
3rd „ „	1·81	1·46	2·12	0·70	1·92	1·00	0·90	0·16
4th „ „	2·35	2·09	—	0·91	1·78	1·22	—	0·21
1st day of Post-period	0·20	1·06	0·91	0·10	1·01	0·52	—	0·09
2nd „ „	0·08	0·30	0·00	0·09	0·00	0·00	1·43	0·09
3rd „ „	0·00	0·00	—	0·00	0·00	0·00	0·38	0·00
4th „ „	0·00	0·00	0·02	0·00	(1·46)	0·00	—	0·06
Totals	10·21	7·57	3·73	2·96	7·39	4·33	3·38	0·80
		11·30			(8·85)	7·71		
Ingested	9·42	12·60		15·10	8·38	12·68		13·70
Balance*	—0·79	+1·30		+12·14	+0·99 (—0·47)	+4·97		+12·90

*A + sign indicates retention by the subject, a — sign indicates loss.

So far as the other metabolites enumerated in the following tables are concerned, the basal output is computed as the average of the outputs on the four days of the fore-period, except in the case of uric acid in which the higher output of the first day, representing delayed excretion consequent upon previous ingestion of purines, is rejected. In the case of the faecal phosphate output of this

subject, however, considerable difficulty is created by the irregularity with which faeces were passed, an irregularity which was attributable in large measure to the nature of the diet. In consequence of the frequent retention of faeces for a day, the *percentage* of phosphates in the faeces probably presents a fairer index of excess excretion than the absolute amount passed on any day, since the latter figure varied with the duration of retention and completeness of evacuation. 5% of P_2O_5 was assumed to represent the basal level of faecal phosphate output, since this was the lowest percentage of P_2O_5 in any sample of faeces passed (on the third day of the first post-period, corresponding to food partaken on the second day of the post-period, see Table 1). On this basis the excess faecal phosphate output was about the same for both types of nucleic acid. The absorption of the two types of nucleic acid from the alimentary canal was therefore about equal.

In the case of the vegetable nucleic acid, the whole of the nitrogenous input was somewhat more than accounted for in the urinary output during the period of ingestion and the succeeding four days, so that there was a negative nitrogen balance. In the post-period following upon the animal nucleic acid period there was a sudden rise of nitrogenous output in the fourth day, following upon two days of subnormal output. The origin of this rise is uncertain. It may represent fluctuation of appetite, or it may represent a sudden release of stored-up products of nucleic acid metabolism, but it is significant that if the defects of excretion during the preceding two days are added together they total 1·34 grms., which is almost exactly the excess encountered on the succeeding day. Possibly, therefore, this nitrogen was not derived from the ingested nucleic acid, in which case only 88% of the ingested nitrogen is accounted for. If the excess nitrogen excreted on the fourth day of the post-period be regarded as derived from the nucleic acid ingested four or more days previously, however, then the whole, and somewhat more than the whole, of the ingested nitrogen is accounted for, as in the case of the vegetable nucleic acid, but the delayed excretion represents a much larger proportion of the total, and the negative balance is smaller, that is, retention is greater in the case of animal than in the case of vegetable nucleic acid.

The urinary excretion of P_2O_5 corresponded to 60% of that ingested in the case of vegetable nucleic acid and only 34% of that ingested in the case of animal nucleic acid. Adding the faecal excretion of phosphates, the total output was 90% of that ingested in the case of vegetable nucleic acid, only 61% in the case of animal nucleic acid. There was, therefore, a very considerable retention of phosphoric acid following upon ingestion of animal nucleic acid, and as in the case of nitrogen the retention of P_2O_5 was greater with animal than with vegetable nucleic acid.

The difference in the behaviour of the two types of nucleic acid, following upon their ingestion, however, is more strikingly evident in the comparative outputs of uric acid than in any other particular, for while ingestion of a total

of 60 grms. of vegetable nucleic acid led to the excretion of an excess of 2·96 grms. of uric acid, or 20% of the theoretical yield from the purine bases of the nucleic acid, ingestion of the same amount of animal nucleic acid led to excess excretion of only 0·80 grms. of uric acid, or 5·8% of the theoretical yield.

In the case of C.S.H. (first experiment) there was some difficulty in assigning a basis for estimation of the nitrogen balance, because this subject started with a very low nitrogenous intake, which was subsequently amended. In

TABLE 3. SUBJECT C.S.H. EXPERIMENT 1.

Date	Volume cc.	Ammonia Nitrogen Grammes	Urea Nitrogen Grammes	Total Nitrogen Grammes	Creatinine Grammes	Uric Acid Grammes	Chlorides as NaCl Grammes	P_2O_5 Grammes	Faecal P_2O_5 Grammes
Purine-free Period.									
30.XI.26	680	0·36	7·55	9·06	1·90	0·36	3·10	1·95	1·15
1.XII.26	550	0·32	5·55	6·80	1·80	0·43	4·27	3·23	1·04
2.XII.26	720	0·24	8·72	9·60	2·08	0·35	4·34	2·76	1·58
3.XII.26	700	0·53	11·20	12·73	2·02	0·36	3·86	2·53	1·33
Animal Nucleic Acid Period.									
4.XII.26	700	0·66	12·10	14·00	2·25	0·63	4·60	2·87	1·73
5.XII.26	825	0·69	13·60	16·13	2·37	0·56	6·11	2·99	3·12
6.XII.26	710	0·63	14·80	16·50	2·20	0·68	6·86	3·36	3·50
7.XII.26	870	0·45	13·30	15·80	2·25	0·56	6·78	3·33	3·45
Purine-free Period.									
8.XII.26	1,060	0·70	14·13	16·30	2·20	0·77	6·74	4·37	2·37
9.XII.26	770	0·42	9·62	10·62	1·90	0·38	4·17	2·40	1·77
10.XII.26	730	0·36	8·53	9·73	1·90	0·36	3·10	2·07	1·03
11.XII.26	920	0·46	10·10	12·60	2·00	0·40	3·95	2·88	1·07
Vegetable Nucleic Acid Period.									
12.XII.26	700	0·46	10·57	12·17	2·40	0·70	4·56	2·40	2·16
13.XII.26	910	0·48	11·70	14·00	2·16	0·60	7·00	3·33	3·67
14.XII.26	1,860	0·62	18·60	20·70	2·15	1·48	7·53	4·28	2·25
15.XII.26	1,365	0·59	12·40	14·80	2·21	1·09	6·21	3·91	2·30
Purine-free Period.									
16.XII.26	750	0·25	8·10	10·00	2·10	0·49	4·71	2·87	1·84
17.XII.26	820	0·25	9·65	11·30	2·00	0·52	3·99	2·53	1·36
18.XII.26	880	0·32	9·77	11·70	2·20	0·46	3·24	1·73	1·51
19.XII.26	900	0·37	10·60	12·50	1·87	0·48	3·38	2·00	1·38

consequence of this the nitrogenous intake and output rose during the last three days of the preliminary purine-free period (see charts in figure 2 and Tables 3 and 4). By the fourth day of the pre-period, however, when the subject had become accustomed to the diet, the intake had reached a steady level, and the output corresponding closely with that attained on the fourth days of both post-periods. The basal output of nitrogen on the purine-free diet was therefore estimated to be 12·5 grms. per diem, and, as it happened when the experiment was subsequently repeated upon this subject, this figure also represented exactly

TABLE 4. SUBJECT C.S.H. EXPERIMENT 2.

Date	Volume cc.	Titratable Acidity in cc. tenth normal alkali per day	Ammonia Grammes	Urea + Ammonia Nitrogen Grammes	Total Nitrogen Grammes	Creatinine Grammes	Uric Acid Grammes	P_2O_5 Grammes	Faecal P_2O_5 Grammes
Purine-free Period.									
25.IV.27	850	408	0·74	10·4	11·9	1·57	0·430	2·13	1·39
26.IV.27	695	480	0·71	10·6	12·2	1·40	0·282	2·59	0·70
27.IV.27	695	480	0·71	10·6	12·2	1·40	0·282	2·59	1·65
28.IV.27	1,110	422	0·66	11·4	13·5	1·60	0·317	2·67	0·65
Animal Nucleic Acid Period.									
29.IV.27	1,670	468	0·77	13·5	14·6	1·56	0·391	3·06	2·18
30.IV.27	970	653	1·02	10·7	12·6	1·70	0·498	3·58	2·65
1.V.27	1,310	647	1·01	12·2	13·5	1·62	0·623	3·84	3·31
2.V.27	1,120	582	0·88	11·3	12·6	1·53	0·623	3·33	2·06
Purine-free Period.									
3.V.27	1,170	575	0·88	10·6	12·0	1·64	0·548	3·50	1·02
4.V.27	780	437	0·73	10·1	11·7	1·68	0·434	2·84	1·52
5.V.27	680	449	0·69	9·9	11·6	1·60	0·340	2·66	1·58
6.V.27	895	510	0·72	10·5	11·7	1·68	0·428	3·07	0·97
Vegetable Nucleic Acid Period.									
7.V.27	880	484	0·81	10·6	11·6	1·55	0·429	2·89	1·08
8.V.27	1,270	664	0·93	10·2	11·4	1·56	0·650	3·41	1·10
9.V.27	1,110	863	1·48	11·3	12·8	1·60	1·041	4·73	2·04
10.V.27	1,000	763	1·05	10·1	11·4	1·65	0·631	3·98	1·50
Purine-free Period.									
11.V.27	890	561	0·86	10·1	11·8	1·58	0·427	3·31	0·80
12.V.27	770	468	0·73	9·7	10·9	1·60	0·342	2·46	0·83
13.V.27	820	500	0·71	10·2	12·0	1·55	0·262	2·70	0·74
14.V.27	775	448	0·63	10·2	11·7	1·65	0·236	2·82	0·58

the average of the outputs on the four days of the pre-period in that experiment. Since the intake in this latter experiment was regulated by appetite, and the initial fluctuations in the first experiment represented progressive adjustments to appetite, we may with some confidence assume that this estimate of the basal output is approximately correct.

Whether we assume the basal output of nitrogen to have been 12·5 grms. or less, however, it remains evident that in this subject a negative nitrogen balance was maintained throughout the duration of the experiment. On the assumed basis the loss of nitrogen during the animal nucleic acid period and the post-period which followed it was 7·95 grms., while during the plant nucleic acid period and the post-period which followed the loss was 2·58 grms. If we take the basal output at the lower figure indicated by the average of the pre-period outputs these losses would be much greater, but the loss during the vegetable nucleic acid period would, of course, continue to be less than the loss during the animal nucleic acid period. The relationship of nitrogen output to the type of nucleic acid ingested was therefore the opposite of that observed in subject T.B.R.

With the subject C.S.H. the regularity of evacuation permitted a steady observation of the faecal phosphate output which was not possible in the subject T.B.R. The excess output of urinary phosphates amounted to 32% of the input in the case of animal nucleic acid, and 31% in the case of vegetable nucleic acid. There was not that disproportion, therefore, between the urinary phosphate outputs with the two types of nucleic acid which was observed in subject T.B.R. The total excess output, urinary and faecal, was 97% of the intake in the case of animal nucleic acid, and 80% in the case of vegetable nucleic acid. With the phosphates, as with the nitrogen, therefore, the results are the opposite of those obtained with subject T.B.R., retention being greater with vegetable than with animal nucleic acid.

When, however, we consider the uric acid excretion the two cases are parallel, and substantially the same results are obtained. Much more uric acid was excreted during the vegetable nucleic acid period and subsequent post-peroid than during the animal nucleic acid period and subsequent post-period. The total excess output arising from vegetable nucleic acid was 18% of the theoretical yield, while that arising from animal nucleic acid was only 9·6% of the theoretical yield.

A totally unanticipated result of the ingestion of both kinds of nucleic acid by these subjects was an increase, slight but very definite, of the apparent output of creatinine (see figures 1 and 2). This occurred in both subjects, and in both the characteristics of the effect were the same. The creatinine output rose in the first twenty-four hours to a value about 15% above the basal output. It stayed at this level throughout the period of ingestion, and then slowly sank, reattaining basal value towards the end of the succeeding purine-free period.

The maximum output of creatinine was somewhat less with animal than with vegetable nucleic acid, and it regained the basal value more quickly during the following period of purine-free diet.

For estimating the creatinine, however, picric acid was employed which had been repeatedly recrystallized from water, but not from benzene. In the subsequent experiments, about to be described, the picric acid which was employed for the creatinine estimations had been repeatedly recrystallized from benzene (3). With this picric acid no rise of creatinine output consequent upon nucleic acid ingestion could be observed. On the contrary, while C.S.H. shows a slight irregular vacillation of creatinine output (figure 2), the other subject of the second experiment, H.R.M., illustrates the classical uniformity of excretion of this metabolite (figure 3). We must conclude, therefore, that the ingestion of nucleic acid leads to an increased output of those substances of unknown nature which react with incompletely purified picric acid, and that the apparent rise of creatinine values during the nucleic acid periods in the first two experiments was entirely attributable to these substances.

The contradictory results in respect to nitrogen and phosphate output with the first two subjects led to a repetition of the experiment. It appeared possible that subject C.S.H. was in an abnormal condition during the first experiment, since he suffered a loss of nitrogen throughout the duration of the experiment, and experienced subjective symptoms of distress which will be described more fully later on. The experiment was therefore repeated upon that subject nearly five months later, and also upon another subject, H.R.M.

In the second experiment with C.S.H. all food consumed was weighed, but freedom of relative choice was maintained. The nitrogen intake was therefore variable, but the relative values could be computed from standard tables. These estimated intakes of nitrogen on each day of the experiment are given in the accompanying table; such estimates are, of course, to be taken as relative only. The "nitrogen balance" depicted upon the graphs in figure 2 represents the difference between these estimates of intake and the actual output, reckoned as positive when the estimated intake exceeded the output.

C.S.H., Second Experiment.

Estimated Nitrogen Intake in Grammes.

	Pre-period.	Animal Nucleic Acid Period.	Post-period.	Vegetable Nucleic Acid-Period.	Post-period.
First day ..	14·1	13·8	12·4	11·8	12·9
Second day ..	15·5	13·2	14·0	13·8	14·4
Third day ..	13·3	12·3	12·1	10·8	13·3
Fourth day..	12·4	15·4	10·6	15·2	12·3
Averages ..	13·8	13·7	12·3	12·9	13·2

Contrary to the previous experiment, the nitrogen balance (estimated from the basal output during the four days of the pre-period) was positive throughout the duration of the experiment. The retention of nitrogen, however, as before, was greater during the second vegetable nucleic acid period than during the first animal nucleic acid period. The urinary excess output of phosphates amounted to 46% of the intake in the animal nucleic acid period and post-period, and 51% of the intake in the vegetable nucleic acid period and post-period. The faecal phosphate output, contrary to the first experiment, did not greatly exceed the urinary output in the animal nucleic acid period, and was much less than the urinary output in the vegetable nucleic acid period. Assimilation of the ingested nucleic acid was therefore much more complete in the second experiment than in the first. The total output of phosphates was 99% of the intake for the animal nucleic acid and 62% of the intake for the vegetable nucleic acid. As in the first experiment, therefore, the retention of phosphorus was greater during the period of vegetable nucleic acid ingestion than it was during the animal nucleic acid period.

As before, the uric acid excretion during and following the vegetable nucleic acid period exceeded that obtained in consequence of the ingestion of animal nucleic acid, but not to so great an extent as in the first experiment, the excess excretion of uric acid in the vegetable nucleic acid period and post-period being 11·7% of theoretical, while the excess excreted during the animal nucleic acid period and post-period was 11·1% of theoretical.

In the experiment with H.R.M. all food was weighed, and the intake was kept rigorously constant. The diet eaten by this subject has been set forth in detail in the introductory portion of this article. The uniformity of nitrogenous intake was reflected in the constancy of the basal nitrogenous output (Table 4).

The nitrogen balance was negative during the first or vegetable nucleic acid period and post-period, and positive during the second or animal nucleic acid period and post-period. As with subject T.B.R., therefore, retention was greater in the animal nucleic acid period and post-period than in the vegetable nucleic acid period and post-period.

The excess urinary output of phosphates constituted 69% of the intake in the case of vegetable nucleic acid, and 46% of the intake in the case of animal nucleic acid. The excess faecal phosphates were in both cases considerably less in amount than the excess urinary phosphates, but were greater in amount during the animal nucleic acid period and post-period than during the vegetable nucleic acid period and post-period. The absorption of animal nucleic acid and its products from the alimentary canal may therefore have been somewhat less complete with the animal than with the vegetable nucleic acid. The total excess output of phosphates was 89% of the intake with vegetable nucleic acid, and 72% of the intake with animal nucleic acid. In each case, therefore, retention occurred, but, as in the previous experiment with T.B.R., the retention was greater with animal than with vegetable nucleic acid.

TABLE 5. SUBJECT C.S.H.

Excess Excretion following Ingestion of Nucleic Acid.

Day.	Animal Nucleic Acid. Nitrogen.	P_2O_5. Urine.	P_2O_5. Faeces.	Uric Acid.	Vegetable Nucleic Acid. Nitrogen.	P_2O_5. Urine.	P_2O_5. Faeces.	Uric Acid.
First Experiment.								
1st day of Ingestion ..	1·50	0·25	0·45	0·25	0·00	0·00	0·88	0·32
2nd „ „	3·63	0·37	1·84	0·18	1·50	0·71	2·39	0·22
3rd „ „	4·00	0·74	2·22	0·30	8·20	1·66	0·97	1·10
4th „ „	3·30	0·71	2·17	0·18	2·30	1·29	1·02	0·71
1st day of Post-period	3·80	1·75	1·09	0·39	0·00	0·25	0·56	0·11
2nd „ „	0·00	0·00	0·49	0·00	0·00	0·00	0·08	0·14
3rd „ „	0·00	0·00	0·00	0·00	0·00	0·00	0·23	0·08
4th „ „	0·10	0·26	0·00	0·02	0·00	0·00	0·10	0·10
Totals	16·33	4·08	8·26	1·32	12·00	3·91	6·23	2·78
		12·34				10·14		
Ingested	8·38	12·68		13·70	9·42	12·60		15·10
Balance*	—7·95	+0·34		12·38	—2·58	+2·46		+12·32
Second Experiment.								
1st day of Ingestion ..	2·1	0·56	1·08	0·10	0·00	0·39	0·00	0·14
2nd „ „	0·1	1·08	1·55	0·20	0·00	0·91	0·00	0·36
3rd „ „	1·0	1·34	2·21	0·33	0·30	2·23	0·94	0·75
4th „ „	0·1	0·83	0·96	0·33	0·00	1·48	0·40	0·34
1st day of Post-period	0·0	1·00	0·00	0·25	0·00	0·81	0·00	0·13
2nd „ „	0·0	0·34	0·42	0·14	0·00	0·00	0·00	0·05
3rd „ „	0·0	0·16	0·48	0·05	0·00	0·20	0·00	0·000
4th „ „	0·0	0·57	0·00	0·13	9·00	0·32	0·00	0·000
Totals	3·3	5·88	6·70	1·53	0·30	6·34	1·34	1·770
		12·58				7·68		
Ingested	8·4	12·68		13·70	9·3	12·37		15·10
Balance*	+5·1	+0·10		+12·17	+9·0	+4·69		+13·33

*A + sign indicates retention by the subject, a — sign indicates loss.

As in the other experiments, the output of uric acid was much higher in this subject when vegetable nucleic acid was ingested than when animal nucleic acid was ingested. The excess excretion of uric acid during and following the vegetable nucleic acid period was 18% of theoretical, while during and following the animal nucleic acid period it was only 7·4% of theoretical.

TABLE 6. SUBJECT H.R.M.

Date	Volume cc.	Titratable Acidity in cc. tenth normal alkali per day	Ammonia Grammes	Urea + Ammonia Nitrogen Grammes	Total Nitrogen Grammes	Creatinine Grammes	Uric Acid Grammes	P_2O_5 Grammes	Faecal P_2O_5 Grammes
Purine-free Period.									
25.IV.27	1,150	426	0·92	11·8	14·3	1·77	0·565	1·82	0·886
26.IV.27	775	458	0·95	11·5	13·9	1·75	0·380	2·40	0·734
27.IV.27	850	459	1·06	11·5	13·9	1·79	0·420	2·59	0·701
28.IV.27	840	572	1·10	11·6	13·8	1·77	0·344	2·73	0·724
Vegetable Nucleic Acid Period.									
29.IV.27	1,085	685	1·21	15·1	17·4	1·74	0·639	3·71	1·115
30.IV.27	1,560	884	1·27	15·7	17·6	1·75	0·815	3·73	0·955
1.V.27	1,670	741	1·32	14·8	16·4	1·77	0·931	3·65	0·749
2.V.27	1,580	668	1·40	14·9	16·8	1·76	1·121	3·76	1·172
Purine-free Period.									
3.V.27	940	440	1·29	12·1	13·9	1·74	0·611	2·76	0·823
4.V.27	900	450	1·09	12·3	13·9	1·75	0·471	2·58	1·384
5.V.27	840	395	1·06	12·4	13·8	1·74	0·417	2·00	0·967
6.V.27	1,000	490	1·11	11·8	13·9	1·76	0·449	2·30	0·963
Animal Nucleic Acid Period.									
7.V.27	1,120	575	1·15	12·0	13·8	1·76	0·497	2·45	0·847
8.V.27	1,235	543	1·18	12·2	13·8	1·76	0·603	3·06	1·086
9.V.27	1,320	541	1·30	12·4	14·3	1·74	0·734	3·05	0·648
10.V.27	1,200	576	1·27	12·4	13·8	1·74	0·508	3·14	1·520
Purine-free Period.									
11.V.27	930	451	1·18	11·9	13·7	1·74	0·372	3·05	1·464
12.V.27	1,000	480	1·19	11·8	13·8	1·76	0·364	2·57	1·580
13.V.27	840	437	1·14	11·9	13·4	1·76	0·282	2·16	0·880
14.V.27	800	464	0·94	11·7	13·7	1·76	0·281	2·36	0·850
15.V.27	790	550	1·09	11·8	13·9	1·74	0·282	2·02	—

To sum up the effects of nucleic acids upon excretion, therefore, it is evident that in subjects T.B.R. and H.R.M., who partook of vegetable nucleic acid first and animal nucleic acid later, retention of nitrogen and phosphorus, during the period occupied by the experiment, was greater with animal nucleic acid than when the administered nucleic acid had been derived from yeast, while in two experiments upon subject C.S.H., who partook of animal nucleic acid first and

TABLE 7. SUBJECT H.R.M.

EXCESS EXCRETION FOLLOWING INGESTION OF NUCLEIC ACID.

Day.	Vegetable Nucleic Acid. Nitrogen.	P_2O_5. Urine.	P_2O_5. Faeces.	Uric Acid.	Animal Nucleic Acid. Nitrogen.	P_2O_5. Urine.	P_2O_5. Faeces.	Uric Acid.
1st day of Ingestion ..	3·6	1·71	0·415	0·295	0·0	0·45	0·147	0·153
2nd „ „	3·8	1·73	0·255	0·471	0·0	1·06	0·386	0·259
3rd „ „	2·6	1·65	0·049	0·587	0·5	1·05	0·000	0·390
4th „ „	3·0	1·76	0·472	0·777	0·0	1·14	0·820	0·164
1st day of Post-period	0·1	0·76	0·123	0·267	0·0	1·05	0·764	0·028
2nd „ „	0·1	0·58	0·684	0·127	0·0	0·57	0·880	0·020
3rd „ „	0·0	0·00	0·267	0·073	0·0	0·16	0·180	0·000
4th „ „	0·1	0·30	2·262	0·105	0·0	0·36	0·150	0·000
5th „ „	—	—	—	—	0·0	0·02	—	0·000
Totals	13·3	8·49	2·527	2·702	0·5	5·86	3·327	1·014
		11·02				9·19		
Ingested	9·3	12·37		15·10	8·4	12·68		13·70
Balance*	—4·0	+1·35		+12·40	+7·9	+3·49		+12·69

*A + sign indicates retention by the subject, a — sign indicates loss.

vegetable nucleic acid later, retention of nitrogen and phosphorus was greater with vegetable than with animal nucleic acid. In three experiments out of four the faecal phosphates formed a greater proportion of the total when animal nucleic acid was eaten than when vegetable nucleic acid was eaten, indicating somewhat less complete absorption of animal nucleic acid from the alimentary canal. In the case of subject T.B.R. absorption was approximately the same for both types of nucleic acid. The following table shows the distribution of phosphorus between the urine and faeces in the various experiments:

TABLE 8.

Distribution of Phosphorus between Urine and Faeces.

Subject.	Order in which Nucleic Acids were administered.	Percentage of Ingested Phosphorus excreted in Urine. Vegetable Nucleic Acid.	Urine. Animal Nucleic Acid.	Faeces. Vegetable Nucleic Acid.	Faeces. Animal Nucleic Acid.
T.B.R.	Animal following Vegetable	60	34	30	27
H.R.M.	Animal following Vegetable	69	46	20	26
C.S.H.1.	Vegetable following Animal	31	32	49	65
C.S.H.2.	Vegetable following Animal	51	46	11	53

In every case the excess excretion of uric acid following upon ingestion of nucleic acid was far less than might theoretically have been derived from the purine bases in the nucleic acid, and inspection of the charts shows quite clearly that the remainder of the purine-nitrogen was largely accounted for by an increased output of urea. In every case uric acid excretion was greater with vegetable nucleic acid than with nucleic acid of animal origin. The volume of urine, titratable acidity, and ammonia output were all greater with vegetable than with animal nucleic acid.

OTHER EFFECTS FOLLOWING INGESTION OF LARGE QUANTITIES OF NUCLEIC ACID.

Two subjects (T.B.R. and H.R.M.) experienced a subjective feeling which is best described as one of "strain" or "nervous tension" during the period of vegetable nucleic acid ingestion. The effect was no greater than would be amply accounted for by the slight degree of acidosis which was undoubtedly present, and which culminated on the fourth day, when the subjective symptoms also attained their maximum intensity. During the period of animal nucleic acid ingestion no subjective symptoms or discomfort of any kind were experienced by these subjects.

In the case of C.S.H. the ingestion of 15 grms. of animal nucleic acid per diem was followed by a steady increase of feelings of personal discomfort. In the first experiment this was attributed in part to excessive perspiration in hot weather, to which this subject was unaccustomed, causing corresponding concentration of his urine. Excess of distilled water was drunk, but without any objective result or easing of symptoms until the ninth day (see charts in figure 2), when a sudden rise in excretion was followed by some relief of symptoms. There would seem to have been some delay of excretion.

During the post-period the symptoms of depression abated, and did not recur with the onset of the new experimental period, during which vegetable nucleic acid was ingested. The hot weather had meanwhile abated somewhat, but it is evident from the curves (figure 2) that this could not be responsible for the relatively greater urinary excretion. The subjective sensations were quite different from those experienced with animal nucleic acid. Depression was replaced by stimulation similar to that experienced by the other subjects when partaking of vegetable nucleic acid. By the fifteenth day the stimulation had become akin to a feeling of "high pressure," as though the subject's "nerves" were "highly strung." Attention became acute. There was some smarting of the conjunctiva (not noticed by the other subjects). Despite these subjective symptoms, however, blood pressure and heart rate remained normal.

In the second experiment with C.S.H. the subjective symptoms were similar to those experienced in the first experiment, but the cooler weather removed the factor of high urinary concentration to which the symptoms had previously been

attributed. By the end of the animal nucleic acid period oedema of the conjunctive and puffiness under the eyes were noted, and confirmed by examination of the subject by Dr. R. F. Matters. These signs did not reappear in the vegetable nucleic acid period. The body weight at the end of the animal nucleic acid period had increased by ½ lb., which may have been due to the oedema.

Each of the subjects noted, while vegetable nucleic acid was being eaten, the presence in the urine of an abundance of crystalline material, the amount of which seemed to be out of proportion to the increase in the output of uric acid, as determined by analysis. At the end of the period these crystals appeared to be present in the urine when passed. The crystals were in the form of fine needles, giving the urine a milky appearance and an iridescent sheen when shaken. In the first experiment on C.S.H. at this stage a catheter was passed in order to ascertain whether these crystals had separated during retention of the urine in the bladder, but one hour's collection of urine obtained in this manner proved that the crystals were issuing as such from the ureters. A sample of the crystals collected from the urine of T.B.R. was dissolved in alkali and reprecipitated with excess of acetic acid several times, finally decolourized by boiling with charcoal in alkaline solution, filtering, and reprecipitating. This precipitate, when dry, proved to contain 95% of uric acid. The abundance of crystals is to be accounted for by the fact that not only the *increase* of uric acid output over basal was precipitated, but also, in consequence of the high acidity of the urine, the basal uric acid output as well. In the animal nucleic acid periods little or no precipitate was noted until the urine cooled, when phosphates were deposited.

Tests for albumin in the urine were repeatedly performed, and were uniformly negative. Nevertheless, during the nucleic acid periods Tsuchiya's reagent (an acid alcoholic solution of phospho-tungstic acid) yielded a measurable precipitate which, estimated as if it had actually been albumin, reached a maximum corresponding to about 0·5 parts per 1,000. This precipitate was not obtained with the normal urines of subjects T.B.R. and C.S.H. (H.R.M. was not tested), and undoubtedly represented a product resulting from the ingestion of nucleic acid.

During the vegetable nucleic acid period positive tests for pentose were obtained in the urine of C.S.H. (first experiment), none in the urine of T.B.R. The urine of H.R.M. and that of C.S.H. in the second experiment were not examined for pentoses.

Blood was withdrawn from subject C.S.H. 4½ hours after the midday meal on the fourth day of each four-day period during the second experiment, and the sugar content was determined before and after hydrolysis (by ½ normal hydrochloric acid for one hour at 100°C.) by the colourimetric method of Folin. The following were the results obtained:

TABLE 9. SUBJECT C.S.H., SECOND EXPERIMENT.

Blood Sugar.

Day and Period.	Estimated per cent. of Sugar in Blood. Before Hydrolysis.	Estimated per cent. of Sugar in Blood. After Hydrolysis for 2 hours by ½ normal acid 100°C.
Fourth day of Pre-period	0·082	0·086
Fourth day of Animal Nucleic Acid Period	0·138	0·143
Fourth day of Post-period	0·097	0·097
Fourth day of Vegetable Nucleic Acid Period	0·101	0·101
Fourth day of Post-period	0·097	0·105

Apparently a slight degree of hyperglycaemia prevailed during the period of animal nucleic acid ingestion, which may have been due to the presence in the blood of reducing substances derived from the nucleic acid, or may have been attributable to the acidosis which prevailed during the nucleic acid ingestion. As the acidosis was higher during the vegetable nucleic acid period, if the latter were the true explanation one would have expected to have observed a hyperglycaemia on the fourth day of this period also, but, as the above results show, no such effect was obtained. The idea underlying the estimation of blood sugar before and after hydrolysis by ½-normal acid was that in this way the presence of circulating nucleosides or nucleotides might be detected, but the figures afford no evidence of the presence of such substances in the blood at the times selected for the withdrawal of the specimens for analysis.

Blood counts were taken at intervals during the vegetable nucleic acid period in subject T.B.R., and during the animal nucleic acid period in subject C.S.H. (first experiment), by Dr. Florence McCoy Hill, to whom our thanks are due for this assistance. No abnormalities of erythrocyte or differential leucocyte count were found, and no leucocytosis was observed in either case.

It may be pointed out that the absence of leucocytosis following ingestion of nucleic acid throws a great deal of doubt upon the efficacy of the oral administration of "nuclein" and yeast nucleic acid, which has been recommended in some quarters for combating certain types of infection. On the other hand, the effect of yeast itself in inducing leucocytosis after oral administration has been established by Heintz and Welker (11). We must conclude, therefore, that this effect of ingesting yeast is attributable to some constituent other than nucleic acid.

BEARING OF THESE RESULTS UPON THE URIC ACID PROBLEM.

Previous authors are unanimous in their testimony that purines, whether ingested in the uncombined form, or nucleic acid, or in tissue, are only partially recoverable in the form of uric acid in the urine. Burian and Schur (6)

obtained 46% recovery of urinary purines after taking 1·6 grms. of hypoxanthine. From feeding experiments they concluded that about 50% of the purines present in animal tissues were recoverable as purines from the urine after ingestion, but, as Folin, Berglund, and Derick point out (8), in some cases the recovery was much less, amounting to as little as 25% of the ingested purine. Taylor and Rose (21) recovered only half of the ingested purines from the urine. Frank and Schittenhelm (9) administered thymus nucleic acid in 10 grm. doses per day to three patients for four, four, and two days respectively. Uric acid equivalent to 5·1%, 10·5%, and 41·1% of the ingested purines was recovered from the urine. Dohrn (7) recovered only 9·7% of purines administered in the form of sweetbreads. Givens and Hunter recovered from 14% to 30% of the theoretical yield of uric acid after administration of yeast nucleic acid (10). It is noticeable in comparing the percentage recoveries reported by different authors that those experiments in which the period of administration was brief (one day or one dose), or in which the dose administered was small, obtained the highest proportion of the theoretical yield of uric acid.

The cause of this disappearance of purines has recently been made clear by the discovery of Folin, Berglund and Derick (8) that uric acid, administered intravenously, is rapidly destroyed in the blood-stream itself, only a relatively small proportion of the uric acid appearing as such, or as allantoin, in the urine. The only organ in which uric acid is stored is the kidney itself, and the effect of such storage, if the dosage of uric acid amounts to as much as 100 mg. per kilo body weight, is to cause the kidneys to "swell up to nearly twice their normal size. They become round and hard, and the surface becomes very shiny; all indicating extreme edema." Later, as the concentration of uric acid in the blood falls, due to its destruction therein, a portion of this stored-up uric acid is returned from the kidneys to the blood. Concurrently the oedema of the kidneys diminishes, but, as Folin, Berglund and Derick point out, "Transient as are the visible effects of the uric acid on the kidneys, these might well be sufficient to produce considerable and less transient effects on the function," and, as determinations of non-protein nitrogen in the blood revealed, it took a dog at least two days to recover an adequate kidney function after injection of 60 mg. of uric acid per kilogram of its body weight.

The daily ingestion of purines in our experiments was equivalent to about 4 grms. of uric acid, or 57 mgs. per kilo body weight. Admittedly ingestion by mouth and in the form of nucleic acid is not comparable with intravenous administration of uric acid itself, but if absorption of nucleic acid from the alimentary canal occurs in the form of nucleotides and nucleosides, the purines are at all events protected from destruction before absorption, so that in the course of twenty-four hours or less (since the total daily dose was administered in twelve hours) we may suppose that the whole of the administered purine entered the blood-stream in a free or combined condition. The proportion of

excess phosphates in the faeces shows (Table 8) that in three out of four experiments, at all events, absorption of the administered nucleic acids was tolerably complete, while the nitrogen output shows clearly that the nitrogenous portion of the nucleic acid was generally completely absorbed. Furthermore, in place of a single injection of 60 mg. of uric acid per kilogram we had a continuous input of purines for four days in succession, equivalent to 57 mg. of uric acid per kilo per diem. It appears not improbable, therefore, that some impairment of kidney function may have resulted.

Evidence of such impairment is clearly revealed in the blood uric acid determinations which were made on subject C.S.H. during the course of the second experiment. Blood was withdrawn for analysis on the fourth day of each four-day period about four and a half hours after the midday meal. The following were the results obtained:

TABLE 10. SUBJECT C.S.H.

Blood Uric Acid.

Day and Period.	Mg. Uric Acid per 100 cc. Blood.	Urinary Uric Acid during corresponding 24 hours.
Fourth day Pre-period	2	0·32 grms.
Fourth day Animal Nucleic Acid Period ..	6	0·62 ,,
Fourth day Post-period..	5	0·43 ,,
Fourth day Vegetable Nucleic Acid Period	7	0·63 ,,
Fourth day Post-period	4·5	0·24 ,,

It will be seen that on the fourth day of the animal nucleic acid period the blood uric acid had risen to three times its level on a purine-free diet, and the urinary output was doubled. After four days of subsequent purine-free diet, the blood uric acid had fallen but slightly, although the output had fallen to only 30% above that on a purine-free diet. On the fourth day of the vegetable nucleic acid period the blood uric acid had risen to $3\frac{1}{2}$ times the level of the pre-period, and the output was once more double that on a purine-free diet, but after four days of a purine-free diet, while the blood uric acid was still more than twice as concentrated as it was in the pre-period, the urinary excretion had actually fallen considerably below the level of the pre-period. This appears to indicate quite definitely that the kidneys were not functioning so efficiently at the end of the experiment as they were at the beginning. This in turn suggests an explanation of the otherwise inexplicable fact that the subjects invariably showed greater retention of nitrogen and phosphates in the *second* nucleic acid period, irrespective of the type of nucleic acid administered. It would appear that the intermediate purine-free period of four days was not sufficiently long to admit of full recovery of normal efficiency by the kidneys, so that the excretion

of nitrogenous metabolites and phosphoric acid was not complete during the second nucleic acid period. The production of uric acid itself, however, was so much more rapid or complete from vegetable than from animal nucleic acid as to overcome the delay in excretion to a sufficient extent to permit the appearance of a greater output of uric acid even when vegetable nucleic acid was taken during the second period. On the one hand we appear to have a diminution of function, and on the other an excess of production, which conceivably might or might not compensate for the loss of function, but in the experiments under review appears to have done so. Under such circumstances, however, as will be clear from the following table, the disproportion between the uric acid production from the two types of nucleic acid was much less than when vegetable nucleic acid was administered during the first nucleic acid period. Presumably delay in excretion permitted a greater proportional destruction in the blood-stream. It is probably for this reason that administration of purines for short periods or in small doses leads to a greater proportional recovery of uric acid than administration in larger doses or for longer periods.

TABLE 11.

Percentage Recovery of Purines in the Form of Uric Acid from the Urine.

Subject.	Order in which the types of Nucleic Acid were administered.	Type of Nucleic Acid Administered. Vegetable.	Animal.
T.B.R.	Vegetable followed by Animal	20	5·8
H.R.M.	Vegetable followed by Animal	18	7·4
C.S.H.1.	Animal followed by Vegetable	18	9·6
C.S.H.2.	Animal followed by Vegetable	11·7	11·1

The loss of efficiency of the kidneys during the second period of nucleic acid administration is, however, shown most clearly if we compare the urinary outputs of phosphoric acid in the first and second periods *in terms of the phosphoric acid which was actually absorbed from the alimentary canal.* Presumably the excess of faecal phosphates represented phosphoric acid derived from nucleic acid which had escaped absorption. Of course, it does not follow that the other portions of the nucleic acid molecule were not absorbed, indeed, the nitrogen balances and curves of total nitrogen and urea output indicate that the nitrogen was assimilated more perfectly than the phosphoric acid. If, however, we subtract the faecal phosphates from the total phosphoric acid ingested we have an estimate of the phosphoric acid, free or combined, which actually entered the blood-stream, and we can determine what proportion of this actual intake reappeared in the urine. The following are the results:

TABLE 12.

Percentage of Absorbed Phosphoric Acid (Ingested less Faecal) which was recovered from the Urine after Nucleic Acid Administration.

Subject	Type of Nucleic Acid Administered.	First Nucleic Acid Period.	Type of Nucleic Acid Administered.	Second Nucleic Acid Period.
T.B.R.	Vegetable	85	Animal	47
H.R.M.	Vegetable	86	Animal	63
C.S.H.1.	Animal	92	Vegetable	61
C.S.H.2.	Animal	98	Vegetable	57

It appears to be quite legitimate to conclude from these figures that the excretory efficiency of the kidneys was diminished to an important extent by the administration of either type of nucleic acid in the dosages employed, and that this diminution of efficiency continued to be evident for a period exceeding four days after the discontinuance of administration.

DISCUSSION OF THE RESULTS.

Despite the diminution of the secretory efficiency of the kidneys which, as we have seen, vitiates the comparison of the excretory products of nucleic acids when administered successively to the same subject, the output of uric acid remains invariably greater when vegetable nucleic acid is eaten than when nucleic acid of animal origin takes its place in the diet. This fact admits of two explanations, that is, either the animal nucleic acid is not absorbed to nearly so great an extent as nucleic acid of vegetable origin, or, being absorbed, it is not decomposed into its constituents with the same rapidity.

The relative absorption of the two types of nucleic acid from the alimentary canal is indicated by the excess of faecal phosphates which succeeds the administration of nucleic acid. While, as we have seen (Table 8), the absorption of animal nucleic acid in three experiments out of four was inferior to the absorption of nucleic acid of vegetable origin, yet this inferiority of absorption was in no case sufficient to account for the very much greater inferiority of uric acid production.

In the four experiments taken together the average absorption of phosphoric acid from vegetable nucleic acid was 25% in excess of the absorption of phosphoric acid from animal nucleic acid, but the average production of uric acid from vegetable nucleic acid was 100% in excess of the production from animal nucleic acid. We must therefore adopt the second alternative explanation, namely, that nucleic acid of animal origin is decomposed less rapidly or less completely into its constituents than nucleic acid of vegetable origin. This conclusion recalls the finding of Levene and Medigreceanu (15), to which reference was made in the introductory part of this paper, that the various enzymes of

alimentary and tissue origin decompose animal nucleic acid much more slowly than nucleic acid of vegetable origin. To this extent, therefore, the possibility indicated by their structures is confirmed, that animal and vegetable nucleic acids differ in their behaviour after ingestion.

Incidentally these results throw considerable doubt upon the theoretical foundations of the classical dietetic treatment of gout (18). If the object be to reduce the production of uric acid, animal tissues, for an equal input of nitrogen, are obviously to be preferred to vegetable tissues. Probably the beneficial effects of a vegetarian diet, if any, are attributable rather to a reduction of nitrogenous intake, and consequently of nucleic acid intake as well. Were the nitrogenous intake reduced to a like extent meat would supply a less injurious form of nucleic acid than vegetables.

So far as other metabolites are concerned, the defective efficiency of the kidneys, which is induced by the dosages of nucleic acid employed in our experiments, completely overshadows any other effect of the administration, and we cannot tell whether or to what extent the one nucleic acid may be utilized preferably to the other.

CONCLUSIONS.

1. Nucleic acids of vegetable and animal origins have been successfully administered to the same human subjects, subsisting upon an otherwise purine-free diet.

2. Animal nucleic acid is slightly less well absorbed from the alimentary canal than nucleic acid of vegetable origin, but, estimated by the faecal phosphates, the difference of absorption between the two types of nucleic acid is not great.

3. On the other hand, the output of uric acid after vegetable nucleic acid is far in excess of that excreted after eating nucleic acid of animal origin. It is inferred that nucleic acid of the vegetable type is decomposed more rapidly or more completely into its constituents than nucleic acid of the animal type.

4. The administration of the dosages of nucleic acid employed in these experiments (15 grms. per diem) leads to a demonstrable deficiency of kidney function.

5. The effect of this loss of renal activity is to induce retention of the various metabolites to such an extent as to vitiate comparisons of output when the two types of nucleic acid are successively administered. Only in the case of uric acid was the excess of production with vegetable nucleic acid sufficient to overcome the retention attributable to a previous administration of nucleic acid of the animal type.

6. Administration of nucleic acid of vegetable or animal origin by mouth leads to the appearance in the urine of some substance or substances other than

creatinine, which react with impure picric acid in alkaline solution, but do not react with picric acid which has been purified by recrystallization from benzene.

7. No leucocytosis or alteration of differential white cell count is induced by oral administration of either form of nucleic acid. The effect of yeast in inducing leucocytosis is therefore not attributable to its content of nucleic acid.

8. On an equal nitrogen intake meat is preferable to vegetables if it be desired to reduce the production of uric acid from the diet.

REFERENCES.

1. Abderhalden, E.: "Lehrbuch der physiologischen Chemie," 5 Aufl. Erste Teil, p. 666.
2. Benedict, S. R.: Journ. Biol. Chem., 51 (1922), p. 189.
3. Benedict, S. R.: Journ. Biol. Chem., 54 (1922), p. 239.
4. Benedict, S. R. and Franke, E.: Journ. Biol. Cham., 52 (1922), p. 388.
5. Bieberfeld, J. and Schmid, J.: Zeitschr. f. physiol. Chem., 69 (1909), p. 292.
6. Burian, R. and Schur, H.: Zeitschr. f. physiol. Chem., 38 (1903), p. 336.
7. Dohrn, M.: Zeitschr. f. physiol. Chem., 86 (1913), p. 130.
8. Folin, O., Berglund, H. and Derick, C.: Journ. Biol. Chem., 60 (1924), p. 361.
9. Frank, F. and Schittenhelm, A.: Zeitschr. f. physiol. Chem., 63 (1909), p. 269.
10. Givens, M. H. and Hunter, A.: Journ. Biol. Chem., 23 (1915), p. 299.
11. Heintz, E. L. and Weller, W. H.: Arch. Int. Med., 35 (1925), p. 450.
12. Jones, W.: Amer. Journ. Physiol., 52 (1920), p. 203.
13. Jones, W.: "Nucleic Acids," 2nd ed., London (1920).
14. Levene, P. A.: Journ. Biol. Chem., 53 (1922), p. 441.
15. Levene, P. A. and Medigreceanu, F.: Journ. Biol. Chem., 9 (1911), pp. 65, 375, and 389.
16. Lusk, G.: "Elements of the Science of Nutrition," 3rd ed., Philadelphia (1921).
17. Morris, J. L. and Macleod, A. G.: Journ. Biol. Chem., 50 (1922), p. 55.
18. Osler, W. and McCrae, T.: "Modern Medicine," 3rd ed., London (1926), vol. 3, p. 202.
19. Ringer, M. and Underhill, F. P.: Journ. Biol. Chem., 48 (1921), p. 523.
20. Tannhauser, S. J. and Czoniczer, G.: Zeitschr. f. physiol. Chem., 110 (1920), p. 307.
21. Taylor, A. G. and Rose, W. C.: Journ. Biol. Chem., 14 (1913), p. 419.

BASAL METABOLISM OF MAN ON A DIET RICH IN NUCLEIC ACID

by

C. STANTON HICKS

(From the Department of Human Physiology and Pharmacology, University of Adelaide).

(Submitted for publication 7th August, 1927.)

Some unpublished observations of T. Brailsford Robertson's on the effect of nucleic acids of plant and animal origin upon the growth of mice pointed to a possible involvement of the cellular metabolism, especially in the case of nucleic acid of animal origin, and it was assumed by Robertson that some evidence of alteration of metabolism might show itself in the urinary metabolites in a human experiment.

This latter method of investigation was adopted, and an apparent increase in creatinine excretion established by Robertson and Hicks (3). The experiment was repeated, and at the same time a careful study of the basal metabolism was carried out on one of the subjects.

Details of the main experimental work are published in another paper by Robertson, Hicks and Marston (3), and so far as the results obtained in the present work are concerned, it is only necessary to state that the experimental period of twenty days was divided into five four-day sections. During the first four days the subject was placed on a purine-free diet, which remained the basal diet throughout the whole period of the investigation. During the second four-day period 15 grms. daily of animal nucleic acid prepared from the spleen were ingested in 5 grm. doses, in addition to the non-purine dietary. The third four days were a repetition of the first purine-free period, and in the fourth period 15 grms. daily of vegetable nucleic acid were administered, the final period being a purine-free period only.

Throughout the whole investigation period the subject refrained from physical exertion of a heavy nature. All measurements were made between 7.30 and 8.30 a.m. under the recognized standard conditions, and the subject was weighed daily, without clothes, before the first meal.

The closed circuit oxygen consumption method was used, the apparatus being a spirometer with circulating fan and CO_2 absorption chamber, together

with mechanism for recording respiratory movements upon a smoked drum, after the manner of Krogh (1).

In this way a graphic record of respiration of oxygen consumed, and time, in second intervals, was obtained, thus providing an accurate check which could be studied at leisure.

The purine-free dietary is illustrated by the appended diet-sheet for one day, freedom of selection from among the various ingredients being permitted.

Diet Sheet for 26/6/27.

Substance.	Grms.	Protein. Grms.	Carbohydrate. Grms.	Fat. Grms.	Calories. Total.
Bread	215·5	16·15	113	2·58	447
Butter	46·5	0·46	—	37·2	345
Cheese	63·5	15·55	1·51	20·2	265
Milk	1110·0	35·50	55·5	42·2	755
Egg	165·5	21·40	—	16·5	253
Flour	15·5	1·20	11·00	0·01	38
Sugar	30·0	—	30·0	—	110
Apple	415·0	2·40	102·0	4·0	456
Potato	277·5	4·7	49·8	—	227
				Grand Total	2,896

Note. Average daily calorific intake during experiment = 2,400 cal. Analytical data taken from Lusk (2).

Nitrogen Intake per Day.

	Purine-free Pre-period. Grms.	Animal Nucleic Acid Period. Grms.	Purine-free Pre-period. Grms.	Vegetable Nucleic Acid Period. Grms.	Purine-free Post-period. Grms.
	14·1	13·8	12·4	11·8	12·9
	15·5	13·2	14·0	13·8	14·4
	13·3	12·3	12·1	10·8	13·3
	12·4	15·4	10·6	15·2	12·3
Average	13·8	13·7	12·3	12·9	13·2

Oxygen Consumption and Basal Calorific Requirements.

Weight, 164 lbs. Height, 5 ft. 9¾ inches. Area, 1·94 sq.m. (Du Bois Formula).

Date.	Barometer (Corrected).	Temperature of Gas °C. (Average).	Time in 6 second Intervals.	Time (Average in Minutes).	Calories per sq. metre per hour.	% of Normal (37·7 cal.).	Remarks.
April 28.	743·4	28·45	32·5	3·25	38·9	+3·1	
			32·2				
			32·5				Purine free.
			32·8				
„ 29.	741·0	26·20	33·8	3·34	38·0	+0·8	
			33·2				
			33·2				
„ 30.	745·2	22·7	33·7	3·35	38·5	+2·1	15 grammes animal nucleic acid per diem.
			33·5				
May 1.	748·5	21·0	34·3	3·47	38·0	+0·8	
			35·1				
„ 2.	747·5	20·0	34·9	3·47	38·1	+1·0	
			34·5				
„ 4.	752·6	27·5	33·1	3·33	38·4	+1·8	
			33·6				
			33·2				
„ 6.	752·6	23·37	34·0	3·30	38·1	+1·0	Purine free.
			32·0				
„ 7.	745·9	24·4	33·4	3·35	38·5	+2·1	
			33·6				
„ 8.	747·0	27·5	32·9	3·30	38·4	+1·8	
			33·3				15 grammes vegetable nucleic acid per diem.
„ 11.	746·2	28·4	30·1	3·18	39·8	+5·9	
			33·0				
			32·3				
„ 12.	753	23·4	36·4	3·68	35·6	—5·6	Purine free.
			37·2				
„ 14.	748	25·0	36·9	3·69	35·0	—4·5	
			38·8				
			35·0				

Note. On May 10, 11, and 12 an attack of influenza supervened. The highest B.M.R. corresponds to the height of the attack; the lower figures following may be due to post-influenzal debility.

The results indicate that within 1 calorie of the average normal for the experimental subject the method would not disclose any alteration in heat

production. After the infection the figures are less reliable, and have been excluded in the determination of the average basal figure, but even these latter results do not fall outside the normal fluctuation in the basal rate.

It is more than probable that the experimental period was too short to detect any alteration in metabolism, which is suggested by the life period results obtained by Robertson with mice, and which will be shortly reported in this journal. The changes in creatinine production, previously referred to, have been shown to be due to some substance which gives Jaffe's test with impure picric acid, and which was increased in amount by the ingestion of nucleic acid (3), so that the present results are in harmony with that conclusion, the creatinine excretion in the present experiment being unaltered when benzene crystallized picric acid was used as the reagent.

My thanks are due to my wife for assistance in securing the basal metabolic measurements.

SUMMARY.

1. Basal metabolism of a human subject on a purine-free diet, with the addition of nucleic acid of plant and animal origin, has been studied.

2. So far as these determinations are concerned, no significant alteration of basal metabolism results from ingestion of nucleic acid, whether of animal or vegetable origin.

BIBLIOGRAPHY.

1. Krogh, A.: Internat. Zeitschr. f. physik.-chemie. Biologie, 1, pp. 491-508.
2. Lusk, G.: "Elements of the Science of Nutrition," 3rd ed., Philadelphia, 1921.
3. Robertson, T. B., Hicks, C. S. and Marston, H. R.: This Journal, vol. 4.

COMPENSATORY RENAL HYPERTROPHY

by

G. R. CAMERON AND C. H. KELLAWAY

(From the Walter and Eliza Hall Institute, Melbourne).

(Submitted for publication 18th July, 1927.)

It is a long-known and well-established fact that after unilateral nephrectomy the surviving kidney undergoes enlargement. A similar enlargement of one kidney also takes places when unilateral hydronephrosis is produced in its fellow by ligation of the ureter. Arataki (1926), who gives an excellent bibliography of the more recent of the numerous papers describing these changes, concludes from his own studies on the white rat, and in agreement with the majority of earlier workers on other species, that the enlargement is a true hypertrophy of the secreting units of the kidney, accompanied by hyperplasia of the supporting tissue, and to some extent of the cells lining the tubules.

The cause of the hypertrophy is still under discussion, and more than one factor is doubtless involved. The surviving kidney has at all events in the early period after the operation an increased blood supply. Sacerdotti (1896) observed no enlargement of the surviving kidney in fasting animals which had been subjected to unilateral nephrectomy, but found that the blood from animals in which both kidneys had been removed caused enlargement of the kidneys when repeatedly injected into normal animals after removing an equivalent amount of blood so as to avoid altering the blood volume. Smith and Moise (1927) have been able to demonstrate increase in the rate of enlargement of the surviving kidney in rats fed on high protein diets, and such diets alone tend in intact rats to cause renal enlargement (Osborne, Mendel, Park, and Winternitz, 1925). It is clear, then, that increased work which has to be done by the surviving kidney is the main factor in causing hypertrophy. It seemed possible, however, that in diseased conditions an added stimulus to hypertrophy might be furnished by a specific stimulant product of the autolysis of damaged renal tissue.

In some experiments on compensatory hypertrophy of the suprarenals in the rabbit, Boycott and Kellaway (1924) attempted to test this general conception by comparison of the effects of excision with those of tying off one adrenal. The results, however, were wholly negative. The comparison of the degree of hypertrophy in animals in which one kidney has been excised with others in which a hydronephrosis has been produced by ligation of the ureter

will not suffice to settle this point, since the stimulus to hypertrophy from piling up of products of metabolism in the blood stream is not necessarily identical in both cases. We have therefore compared the extent of hypertrophy in three series of animals. In one group unilateral nephrectomy was performed in some animals, ligature of one ureter in others, while the remainder were unoperated controls. In the second the results of unilateral nephrectomy were compared with the hypertrophy produced when in addition repeated subcutaneous injections of fresh-minced homologous renal tissue were made. In the final group the extent of hypertrophy following nephrectomy was compared with that which follows the ligation of the pedicle of one kidney, a process which results in complete atrophy on the operated side within 70 days, and does not appear to affect the general health of the animal.

METHODS.

The animals used were guinea-pigs, 158 in number. Group 1 (a) comprised 21 guinea-pigs in which the left kidney had been excised, 13 in which hydronephrosis was produced by ligation of the left ureter, and 14 control animals, in which no operation was performed. These were studied after 60 days. In Group 1 (b), of 27 animals, in 14 the left kidney was removed, and in 13 the left ureter was ligated. The animals were killed 90 days after operation. In all the animals of Group 2 (42 in number) the left kidney was removed, and in 21 of these weekly injections of fresh guinea-pig kidney tissue were given subcutaneously. These were killed 75 to 78 days after operation, except 3 killed on the 95th day. In Group 3 (of 41 guinea-pigs) in 20 the left kidney was removed, and in 21 the vessels and ureter were tied off *en masse* at the hilum. In this group the animals were nearly all killed after 76 days. All these experiments were so arranged that animals in which the results were to be compared came from the same litters.

The operative procedures were carried out by one of us (C.H.K.) under full ether anaesthesia and with aseptic precautions. The animals were from 14 to 30 days old when operated upon, and weighed from 110 to 280 grms. The younger animals were returned to their mothers after operation, and nourished by suckling until a month old. A short incision was made in the left flank, the peritoneum was opened, and the kidney delivered. When the ureter was ligated this was done immediately distal to the pelvis of the kidney. For nephrectomy or simple tying off of the kidney alone the vessels and ureter were ligated *en masse* at the hilum after the capsule had been stripped back, and in the female, before delivering the kidney for decapsulation, it was necessary to divide between ligatures the suspensory ligament of the ovary to avoid post-operative haemorrhage from the artery accompanying it. The time occupied in the operations varied from 7 to 10 minutes, and the immediate mortality was 6·4%. All wounds healed by first intention. When injections of fresh renal tissue were

made, a guinea-pig was killed, the kidneys removed aseptically, minced in a tumour mincer, and injected in doses of 0·05 c.cm. with a Bashford syringe. This procedure was carried out with strict aseptic precautions. The animals were kept, one only in each cage, under uniform conditions, and the diet, which was ample, consisted of bran, cabbage, lettuce leaves, and occasionally carrots.

The temperature of the animal houses was regulated when necessary by steam radiators, and the animals were regularly exposed to sunlight for similar periods.

The guinea-pigs were autopsied in the same order as that in which they had been operated on, controls being killed on the same day as their litter mates. The technique was as follows. After fasting over night for 12–18 hours the animal was weighed, killed by a blow on the head, and then bled out as far as possible by dividing the vessels in the neck. The thoracic and abdominal cavities were opened and the viscera removed, stored in a moist air chamber, and weighed at leisure. The alimentary canal, gall bladder, and urinary bladder were weighed after their contents had been washed out. The final body weight used in calculations was obtained by adding the weight of the organs to that of the eviscerated carcase.

The dry weight of the organs was also obtained by drying them in an oven at 70°C. for 72 hours, and finally in a desiccator to constant weight.

RESULTS.

The results of the experiments are summarized in Tables 1 (a), 1 (b), 2, and 3. In these the weight of the body at death, and the wet and dry weights of the surviving kidney, heart, and liver, are given for each animal, together with the ratios ($\times$ 1,000) of the weights of the organs in grammes to the body weight in grammes. The mean values of the ratios in each group and of the body weights have been calculated, and in the former case the standard deviation and standard error of the mean have been determined wherever the number of observations has not been too small for this purpose.

The operations did not cause any definite symptoms, save in guinea-pig 1, Table 1 (a), which on the 60th day after nephrectomy had a series of fits and a blood urea of 87 mgm. per 100 c.cm. Miss Beryl Splatt determined the blood urea values in 30 animals of Groups 1 (a) and (b) at death. Sixty days after nephrectomy the mean value was 53 mgm. per 100 c.cm. (8 observations), 90 days after nephrectomy, 43 mgm. (8 observations), 60 days after unilateral ligation of the ureter, 55 mgm. (5 observations), and 90 days after ligation of the ureter 47 mgm. (9 observations). The mean value for the blood urea in a series of considerably older normal guinea-pigs was 56 mgm. per 100 c.cm. These results suggest some improvement in function with lengthening of the period of survival, but are too few to be significant, except in so far as they indicate that there was

no gross impairment of function in the animals so tested apart from the case mentioned above.

In Group 1 (a) the mean value of the ratio ($\times$ 1,000) of the wet weight of the surviving kidney to the body weight was 7·04 $\pm$ ·11 in the nephrectomy sub-group, 6·51 in the hydro-nephrotic sub-group, and 4·69 $\pm$ ·10 in the corresponding controls. The mean ratios ($\times$ 1,000) of the dry weight to the body weight were 1·44 $\pm$ ·03, 1·35 and 0·94 $\pm$ ·02, and indicate that there has been no appreciable increase of the water content in the surviving kidney in the hydronephrotic or nephrectomized sub-group. Compensatory hypertrophy of the surviving kidney is definitely greater in the nephrectomized than in the hydronephrotic guinea-pigs at this period after operation. The mean body weights in the three sub-groups, 362, 369, and 365 gms., were so close that a less degree of hypertrophy in the hydronephrotic group cannot be attributed to nutritional causes. In the absence of evidence that the stimulus of increased load on the surviving kidney is greater in the case of animals in which one kidney has been removed, though the kidney may have a somewhat greater vascular supply during the early period after the operation, the observed facts might even be held to suggest a hypothesis diametrically opposite to that which we were testing, namely, that products of renal tissue autolysis actively inhibit hypertrophy of the surviving kidney.

In this group the mean of the values of the ratio ($\times$ 1,000) of the wet weight of the heart to the body weight was 3·50 $\pm$ ·04 in the nephrectomized, 3·36 $\pm$ ·06 in the hydronephrotic, and 3·33 $\pm$ ·07 in the control group. The corresponding dry weight ratios were ·59 $\pm$ ·02, ·59 and ·66 $\pm$ ·02, and indicate a somewhat greater water content in the heart muscle in the two operated sub-groups. These figures clearly show that in young guinea-pigs the compensatory hypertrophy of the surviving kidney, which occurs following removal of one kidney or ligation of one ureter, is unaccompanied by hypertrophy of the heart. Grawitz and Israel (1897), among the earlier workers, noted that hypertrophy of the heart only follows extirpation of one kidney in the case of the older animals.

The mean of the rations ($\times$ 1,000) of the wet weight of the liver to body weight was 31·33 $\pm$ ·46 in the nephrectomized sub-group, 29·69 $\pm$ 1·46 in the hydronephrotic, and 30·00 $\pm$ ·77 in the control sub-group. The corresponding means of the dry weight ratios were 7·30 $\pm$ ·33, 7·30 and 7·00 $\pm$ ·25.

The results of Group 1 (b), examined 90 days after operation, confirm the findings in Group 1 (a). The mean ratios ($\times$ 1,000) of wet weight of the surviving kidney to body weight were 6·00 $\pm$ ·14 in the nephrectomized, and 5·71 $\pm$ ·08 in animals after ligation of one ureter, the corresponding dry weight ratios being 1·15 $\pm$ ·01 and 1·10 $\pm$ ·02. The difference between these values, unlike the observed difference at the 60th day, is only just outside the standard error, and renders it probable that the larger difference at the earlier period is due to greater vascularity in and possibly increased functional demand

upon the surviving kidney of the nephrectomized animals in the early days after operation. The means of the wet ratios (× 1,000) of the heart to body weight were 3·07 ± ·06 and 2·96 ± ·03, and those of the liver were 29·14 ± ·95 and 26·62 ± ·84.

In Group 2 an attempt has been made to evaluate the effect of the subcutaneous injection of fresh sterile renal tissue on the compensatory hypertrophy of the surviving kidney. In the control sub-group, from which the left kidney had been removed, but no injections made, the means of the wet and dry weight ratios (× 1,000) were 7·21 ± ·16 and 1·56 ± ·03 for the surviving kidney, as compared with the sub-group in which removal of the left kidney was followed by a series of injections of renal tissue, and in which the values were 7·09 ± ·16 and 1·52 ± ·03. The difference observed is within the standard error of the mean, so that no effect has been demonstrated. That no impairment of the general health or nutrition of the animals was caused by the injections is indicated oy the mean body weights, which were 347 in the uninjected and 351 in the injected sub-group of guinea-pigs. It might be argued that the dosage of renal tissue injected (·05 c.cm weekly) was too small to produce any result. A final experiment was therefore made in Group 3, in which the same comparisons were instituted between a sub-group in which nephrectomy had been performed and another in which the pedicle of the left kidney had been tied off *en masse* and the organ left *in situ*. These last animals, examined after 76 days, had almost the same mean body weight (439 grms.) as that of the guinea-pigs in which the kidney had been removed after ligation of the pedicle (436 grms.). At this time the tied-off kidney had atrophied, so that about 1 grm. (the average weight of the left kidney when removed at operation) of renal tissue had been autolyzed and absorbed during this period.

In the control group of nephrectomized animals the means of the ratios (× 1,000) of wet and dry weights to body weight were 7·20 ± ·10 and 1·70 ± ·05 for the surviving kidney as compared with the corresponding values in the groups in which the kidney was tied off, 6·96 ± ·07 and 1·62 ± ·03. No significant difference is detectable here.

Whatever influence products of autolysis of differentiated tissue may have in causing new growth of fibrous tissue, it is clear that they neither increase nor decrease the hypertrophy of the surviving kidney. The figures in Table 3 likewise exclude the possibility of their causing hypertrophy of the heart or liver.

CONCLUSIONS.

1. Specific products of autolysis of renal tissue have no influence in causing compensatory overgrowth of the surviving kidney in the guinea-pig following unilateral nephrectomy or ligation of a single ureter.

2. No cardiac or hepatic overgrowth is observed in young guinea-pigs following these procedures.

REFERENCES.

1. Arataki, M.: Amer. Journ. Anat., 36 (1926), p. 437; 38, p. 71.
2. Boycott, A. E. and Kellaway, C. H.: Journ. Path. and Bact., 27 (1924), p. 171.
3. Grawitz, P. and Israel, O.: Virchow's Arch., 77 (1897), p. 315.
4. Osborne, T. B., Mendel, L. B., Park, E. A. and Winternitz, M. C.: Amer. Journ. Physiol. Proceed., 72 (1925), p. 222.
5. Sacerdotti, C.: Virchow's Archiv., 146 (1896), p. 267.
6. Smith, A. H. and Moise, T. S.: Journ. Exper. Med., 45 (1927), p. 263.

SERIES 1.
Group 1 (a).
Sixty Days.

Excision of Left Kidney.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Grammes.											
					Kidney.				Heart.				Liver.			
					Weight.		Ratio (× 1,000).		Weight.		Ratio (× 1,000).		Weight.		Ratio (× 1,000).	
					Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.
1	I	M	60	498	2·87	—	5·76	—	1·68	—	3·37	—	14·83	—	29·78	—
3	II	M	60	436	3·01	—	6·96	—	1·77	—	4·06	—	13·26	—	30·41	—
4	II	F	60	353	2·40	—	6·81	—	1·20	—	3·40	—	10·22	—	28·95	—
6	III	M	60	339	2·20	—	6·50	—	1·16	—	3·42	—	9·68	—	28·56	—
8	IV	M	60	432	2·72	—	6·30	—	1·47	—	3·41	—	13·22	—	30·60	—
9	IV	M	60	415	2·77	—	6·68	—	1·31	—	3·16	—	13·12	—	31·61	—
39	XVIII	F	65	416	2·45	0·509	5·89	1·22	1·31	0·212	3·15	0·51	13·56	4·220	32·59	10·14
43	XIX	F	65	395	2·58	0·541	6·53	1·37	1·36	0·234	3·44	0·59	13·77	4·400	34·86	11·14
46	XIX	M	65	332	2·29	0·388	6·89	1·17	1·23	0·155	3·71	0·47	11·18	2·986	33·67	8·99
51	XXI	F	60	276	2·10	0·413	7·61	1·50	0·90	0·151	3·26	0·55	7·96	1·599	28·84	5·79
52	XXII	F	60	296	2·54	0·550	8·58	1·86	0·98	0·106	3·31	0·36	7·97	1·861	26·93	6·29
56	XXIII	M	60	402	2·85	0·551	7·09	1·37	1·35	0·235	3·36	0·59	11·88	2·232	29·55	5·55
57	XXIV	F	60	340	2·58	0·547	7·59	1·61	1·15	0·221	3·38	0·65	10·76	2·138	31·65	6·29
63	XXVI	M	60	320	2·30	0·468	7·19	1·46	1·10	0·210	3·44	0·66	9·54	1·636	29·81	5·11
66	XXVIII	F	60	360	2·38	0·466	6·61	1·29	1·23	0·236	3·42	0·66	11·88	2·138	33·00	8·94
69	XXIX	M	60	314	2·48	0·502	7·89	1·57	1·02	0·212	3·25	0·68	11·88	2·561	37·83	8·16
72	XXX	F	60	325	2·18	0·416	6·71	1·28	1·23	0·238	3·78	0·37	8·78	1·520	27·02	4·68
73	XXX	M	60	366	2·50	0·513	6·83	1·40	1·32	0·238	3·61	0·65	12·88	2·768	35·19	7·56
75	XXXI	F	60	337	2·37	0·479	7·03	1·42	1·32	0·268	3·92	0·80	11·55	2·613	34·27	7·75
76	XXXI	M	60	324	2·63	0·525	8·12	1·62	1·20	0·255	3·71	0·79	9·74	1·876	30·06	5·790
79	XXXII	F	60	328	2·68	0·522	8·17	1·59	1·21	0·252	3·69	0·77	12·17	2·468	37·10	7·52
Mean				362			7·04	1·44			3·50	0·59			31·33	7·30
Standard Deviation							0·729	0·182			0·247	0·122			3·106	1·91
Probable Error of Mean							±0·11	±0·03			±0·04	±0·02			±0·46	±0·33

Group 1 (a) (continued).
Sixty Days.

Ligation of Left Ureter.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Grammes.											
					Kidney.				Heart.				Liver.			
					Weight.		Ratio (× 1,000).		Weight.		Ratio (× 1,000).		Weight.		Ratio (× 1,000).	
					Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.
2	I	M	60	425	2·79	—	—	—	1·54	—	3·63	—	12·11	—	28·49	—
5	II	M	60	410	2·65	—	—	—	1·55	—	3·78	—	13·01	—	31·73	—
7	III	M	60	357	2·39	—	—	—	1·20	—	3·36	—	9·59	—	26·86	—
10	IV	M	60	382	2·51	—	—	—	1·22	—	3·19	—	11·37	—	29·76	—
40	XVIII	F	65	330	2·18	0·433	6·61	1·31	1·06	0·169	3·22	0·51	9·46	2·801	28·67	8·49
44	XIX	F	65	306	1·90	0·392	6·21	1·28	1·06	0·118	3·47	0·39	7·52	1·488	24·57	4·86
48	XX	M	60	421	2·37	0·502	5·63	1·19	1·32	0·152	3·13	0·36	10·65	2·784	25·29	6·61
50	XXI	F	60	345	2·39	0·512	6·93	1·48	1·32	0·261	3·83	0·76	9·75	2·012	28·26	5·83
53	XXII	F	60	386	2·48	0·538	6·43	1·39	1·20	0·196	3·11	0·51	10·17	2·318	26·35	6·01
54	XXIII	F	60	350	2·25	0·496	6·43	1·42	1·07	0·188	3·06	0·54	9·57	2·088	27·34	5·68
58	XXIV	F	60	343	2·49	0·528	7·26	1·54	1·22	0·241	3·56	0·70	11·56	2·324	33·70	6·78
59	XXV	F	60	384	2·45	0·488	6·38	1·27	1·45	0·276	3·78	0·79	14·17	3·006	36·90	7·83
61	XXVI	F	60	355	2·21	0·478	6·23	1·35	0·99	—	2·79	—	12·72	2·586	35·83	7·28
Mean				369			6·51	1·35			3·36	0·59			29·69	7·30
Standard Deviation											0·329				3·667	
Probable Error of Mean											±0·06				±1·464	
Controls: Right Kidney.																
41	XVIII	M	65	428	1·87	0·386	4·37	0·90	1·25	0·220	2·92	0·51	14·30	4·495	33·41	10·50
42	XIX	F	65	400	1·44	0·293	3·60	0·73	1·05	0·284	2·63	0·71	9·59	2·930	23·98	7·33
45	XIX	M	65	275	1·27	0·261	4·62	0·95	0·99	0·172	3·59	0·63	7·87	2·312	28·62	8·41
47	XX	M	60	438	1·71	0·351	3·90	0·80	1·30	0·188	2·97	0·43	11·12	2·986	25·39	6·59
49	XXI	F	60	383	1·69	0·382	4·38	1·00	1·20	0·302	3·14	0·79	11·22	2·518	29·29	6·78
55	XXIII	M	60	356	1·70	0·372	4·77	1·05	1·08	0·210	3·03	0·59	8·90	1·887	25·00	5·30
60	XXV	M	60	444	2·00	0·389	4·50	0·88	1·62	0·336	3·65	0·76	16·00	3·868	36·04	8·71
62	XXVI	M	60	332	1·80	0·360	5·42	1·08	1·06	0·202	3·19	0·61	10·12	2·005	30·48	6·04

62	XXVI	M	60	332	1·80	0·360	5·42	1·08	1·06	0·202	3·19	0·61	10·12	2·005	30·48	6·04

Group 1 (a) (continued).

Controls: Right Kidney (continued). Sixty Days.

Number of Animal.	Number of Litter.	Sex.	Time after Operation. Body Weight at Death.		Kidney. Weight. Wet.	Kidney. Weight. Dry.	Kidney. Ratio (×1,000). Wet.	Kidney. Ratio (×1,000). Dry.	Heart. Weight. Wet.	Heart. Weight. Dry.	Heart. Ratio (×1,000). Wet.	Heart. Ratio (×1,000). Dry.	Liver. Weight. Wet.	Liver. Weight. Dry.	Liver. Ratio (×1,000). Wet.	Liver. Ratio (×1,000). Dry.
			Weight of Organs in Grammes with their Ratios (×1,000) to Body Weight in Grammes.													
65	XXVII	M	60	375	1·85	0·352	4·93	0·94	1·15	0·246	3·07	0·66	11·58	2·112	30·88	5·63
68	XXIX	M	60	349	1·90	0·328	5·44	0·94	1·16	0·218	3·33	0·63	12·86	2·488	36·85	7·13
70	XXX	M	60	342	1·58	0·312	4·62	0·91	1·30	0·261	3·80	0·76	8·79	1·762	25·70	5·15
74	XXX	F	60	339	1·50	0·299	4·42	0·88	1·22	0·224	3·60	0·66	10·76	2·132	31·74	6·29
78	XXXI	F	60	327	1·78	0·326	5·44	0·10	1·29	0·251	3·95	0·77	10·36	2·068	31·68	6·32
80	XXXII	F	60	319	1·68	0·326	5·26	1·02	1·20	0·236	3·77	0·74	9·52	1·718	29·84	5·39
Mean				365			4·69	0·94			3·33	0·66			30·00	7·00
Standard Deviation							0·549	0·084			0·382	0·093			4·278	1·40
Probable Error of Mean							±0·10	±0·02			±0·07	±0·02			±0·77	±0·25

Group 1 (b).

Excision of Left Kidney. Ninety Days.

11	V	F	93	432	2·41	0·465	5·58	1·08	1·25	0·198	2·89	0·46	13·02	3·982	30·14	9·22
12	V	M	93	471	2·50	0·515	5·31	1·09	1·49	0·268	3·17	0·57	13·85	3·774	29·41	8·01
15	VI	M	93	496	2·75	0·515	5·55	1·04	1·68	0·230	3·38	0·46	14·21	4·340	28·65	8·75
17	VII	M	93	461	2·41	0·472	5·23	1·02	1·14	0·197	2·47	0·43	11·39	3·478	24·71	7·54
18	VIII	M	92	387	2·91	0·542	7·52	1·40	1·48	0·237	3·83	0·61	14·02	4·250	36·23	10·78
22	IX	M	92	340	2·70	0·442	7·94	1·30	1·22	—	3·59	—	13·20	3·694	38·82	10·87
23	XIII	M	82	470	3·17	0·505	6·74	1·08	1·42	0·260	3·06	0·52	10·59	3·051	23·75	6·88
24	X	M	92	464	2·44	0·467	5·32	1·01	1·42	0·240	2·88	0·47	11·02	3·194	27·65	7·07
27	XI	F	92	340	2·23	0·426	6·56	1·25	0·98	0·161	3·05	0·50	9·40	2·402	21·81	6·15
28	XII	F	92	442	2·61	0·522	5·91	1·18	1·35	0·223	3·02	0·55	9·64	2·718	22·53	6·49
31	XIV	F	92	468	2·68	0·556	5·73	1·19	1·45	0·290	3·09	0·63	11·85	3·725	25·32	7·96
33	XV	F	92	481	2·69	0·582	5·59	1·21	1·33	0·271	2·77	0·56	17·16	5·022	35·67	10·44
35	XVI	F	91	430	2·69	0·515	6·26	1·20	1·36	0·172	3·16	0·40	12·38	2·488	28·79	5·79
37	XVII	F	91	470	2·58	0·512	5·49	1·09	1·31	0·246	2·79	0·52	15·38	3·026	32·72	6·44
Mean				439			6·00	1·15			3·07	0·53			29·14	8·00
Standard Deviation							0·782	0·125			0·336	0·074			5·273	1·64
Probable Error of Mean							±0·14	±0·01			±0·06	±0·01			±0·95	±0·29

Group 1 (b) (continued).

Ligation of Left Ureter. Ninety Days.

Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Grammes.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Kidney. Weight. Wet.	Kidney. Weight. Dry.	Kidney. Ratio (× 1,000). Wet.	Kidney. Ratio (× 1,000). Dry.	Heart. Weight. Wet.	Heart. Weight. Dry.	Heart. Ratio (× 1,000). Wet.	Heart. Ratio (× 1,000). Dry.	Liver. Weight. Wet.	Liver. Weight. Dry.	Liver. Ratio (× 1,000). Wet.	Liver. Ratio (× 1,000). Dry.
13	VI	F	93	402	2·15	0·447	5·35	1·11	1·20	0·148	2·98	0·37	11·98	3·552	29·80	8·84
14	VI	M	93	418	2·30	0·432	5·50	1·03	1·20	0·184	2·88	0·44	14·72	3·908	35·22	9·35
16	VII	M	93	500	2·80	0·474	5·60	0·95	1·53	0·148	3·06	0·30	16·51	4·486	33·02	8·97
19	VIII	F	93	406	2·22	0·470	5·47	1·16	1·13	0·192	2·78	0·47	8·87	2·598	21·85	6·40
20	VIII	F	92	388	2·10	0·393	5·41	1·01	1·15	0·206	2·96	0·53	11·65	3·240	30·03	8·35
21	IX	F	93	420	2·47	0·462	5·88	1·10	1·24	0·218	2·95	0·52	9·71	2·925	23·12	6·96
25	X	M	92	505	2·97	0·551	5·88	1·09	1·59	0·272	3·15	0·54	12·10	3·152	23·96	6·24
26	XI	M	92	460	2·58	0·498	5·61	1·08	1·38	0·220	3·00	0·48	9·93	3·073	21·59	6·69
29	XII	M	92	490	2·60	0·535	5·31	1·09	1·38	0·247	2·82	0·48	10·45	3·775	21·33	7·70
32	XIV	M	92	375	2·67	0·510	7·12	1·36	1·20	0·223	3·20	0·60	10·31	2·930	27·49	7·81
34	XV	F	92	394	2·50	0·498	6·34	1·26	1·18	0·208	2·99	0·53	9·98	2·010	25·33	5·10
36	XVI	F	91	490	2·66	0·538	5·43	1·10	1·39	0·237	2·84	0·48	14·77	3·126	30·14	6·38
38	XVII	M	91	530	2·78	0·558	5·25	1·05	1·52	0·218	2·87	0·41	16·88	3·487	31·85	6·58
Mean				444			5·71	1·10			2·96	0·47			26·62	7·27
Standard Deviation							0·451	0·089			0·139	0·092			4·483	1·12
Probable Error of Mean							±0·08	±0·02			±0·03	±0·02			±0·84	±0·21

SERIES II.

Group 2.

Excision of Left Kidney.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Grammes.											
					Kidney.				Heart.				Liver.			
					Weight.		Ratio (× 1,000).		Weight.		Ratio (× 1,000).		Weight.		Ratio (× 1,000).	
					Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.	Wet.	Dry.
82	XXXIII	F	—	412	2·35	0·480	5·70	1·16	1·36	0·244	3·30	0·59	12·76	2·471	30·97	6·00
83	XXXIV	F	—	285	2·02	0·482	7·07	1·68	1·05	0·202	3·69	0·71	9·19	2·063	32·25	7·24
85	XXXV	M	—	363	2·90	0·622	7·99	1·71	1·34	0·235	3·69	0·65	15·22	3·167	41·93	8·72
88	XXXVI	F	—	387	2·79	0·586	6·98	1·53	1·32	0·266	3·41	0·69	13·65	2·448	35·27	6·33
89	XXXVII	F	—	395	2·26	0·535	5·72	1·36	1·40	0·288	3·55	0·73	14·25	3·164	36·08	8·01
94	XXXIX	F	—	320	2·18	0·474	6·81	1·48	1·21	0·254	3·78	0·79	10·50	2·228	32·81	6·96
96	XL	F	—	320	2·30	0·488	7·19	1·52	1·21	0·237	3·78	0·74	12·00	2·586	37·50	8·08
99	XLI	M	—	410	2·77	0·602	6·76	1·47	1·40	0·346	3·42	0·85	12·07	3·122	29·44	7·62
100	XLII	M	—	346	2·67	0·559	7·72	1·62	1·38	0·326	3·99	0·95	13·95	3·264	40·32	9·43
103	XLIII	M	—	365	2·84	0·576	7·78	1·58	1·29	0·312	3·53	0·86	10·07	2·516	27·59	6·89
107	XLIV	F	—	200	2·25	0·440	11·25	2·20	0·90	0·250	4·50	1·25	7·20	2·461	36·00	12·31
108	XLIV	F	—	315	2·25	0·468	7·14	1·48	1·23	0·312	3·91	0·99	9·30	2·336	29·52	7·42
109	XLV	F	—	360	2·38	0·520	6·61	1·45	1·06	0·210	2·95	0·58	10·15	2·388	28·19	6·63
112	XLVI	F	—	322	2·45	0·572	7·61	1·78	1·10	0·232	3·42	0·72	11·55	2·916	35·87	9·06
113	XLVI	M	—	344	2·40	0·540	6·98	1·57	1·12	0·223	3·25	0·65	9·52	2·161	27·67	6·28
116	XLVII	M	—	345	2·40	0·496	6·96	1·44	1·25	0·261	3·62	0·76	12·40	2·812	35·94	8·15
119	XLVIII	F	—	340	2·45	0·598	7·21	1·76	1·26	0·218	3·71	0·64	9·52	1·961	28·00	5·77
121	XLIX	M	—	390	2·39	—	6·13	1·25	1·25	0·258	3·21	0·66	12·48	2·563	32·00	6·57
122	L	F	—	400	2·81	—	7·03	1·44	1·35	0·279	3·38	0·70	15·93	3·324	39·83	8·31
126	LI	M	—	340	2·66	—	7·02	1·59	1·48	0·375	4·36	1·11	13·35	2·688	39·27	7·91
127	LI	F	—	330	2·46	—	7·46	1·87	1·38	0·279	4·18	0·85	14·48	2·988	43·88	9·06
Mean				347			7·21	1·56			3·64	0·78			34·38	7·79
Standard Deviation							1·091	0·222			0·356	0·173			4·764	1·61
Probable Error of Mean							±0·16	±0·03			±0·05	±0·03			±0·70	±0·24

Series II.

Group 2 (continued).

Excision of Left Kidney with Injection of Renal Substance.

Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Grammes.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Kidney. Weight. Wet.	Kidney. Weight. Dry.	Kidney. Ratio (× 1,000). Wet.	Kidney. Ratio (× 1,000). Dry.	Heart. Weight. Wet.	Heart. Weight. Dry.	Heart. Ratio (× 1,000). Wet.	Heart. Ratio (× 1,000). Dry.	Liver. Weight. Wet.	Liver. Weight. Dry.	Liver. Ratio (× 1,000). Wet.	Liver. Ratio (× 1,000). Dry.
81	XXXIII	F	95	350	2·35	0·498	6·71	1·42	1·22	0·216	3·49	0·62	11·78	2·067	33·66	5·91
84	XXXIV	M	95	463	2·85	0·633	6·16	1·37	1·59	0·269	3·44	0·58	14·88	2·885	32·14	6·23
87	XXXVI	F	95	422	2·76	0·598	6·54	1·41	1·34	0·225	3·17	0·53	12·78	2·286	30·28	5·42
90	XXXVII	F	78	387	2·32	0·493	5·99	1·28	1·19	0·243	3·08	0·63	14·36	2·874	37·11	7·43
92	XXXVIII	M	78	403	2·34	0·516	5·81	1·28	1·40	0·237	3·48	0·59	12·22	2·136	30·32	5·30
93	XXXIX	F	77	310	2·28	0·482	7·36	1·56	1·15	0·225	3·71	0·73	10·56	1·989	34·07	6·42
97	XL	F	77	350	2·34	0·486	6·69	1·39	1·17	0·247	3·35	0·71	10·02	2·165	28·61	6·19
98	XLI	F	75	392	2·61	0·556	6·66	1·42	1·29	0·336	3·29	0·86	9·96	2·483	25·41	6·33
101	XLII	M	75	360	2·72	0·548	7·56	1·56	1·14	0·361	3·17	1·00	10·88	2·862	30·22	7·95
102	XLII	M	75	224	2·41	0·494	10·76	2·20	0·97	0·244	4·33	1·09	6·90	1·682	30·80	7·51
104	XLIII	M	75	320	2·48	0·512	7·75	1·60	1·24	0·312	3·87	0·98	9·25	2·386	28·91	7·46
106	XLIV	F	75	345	2·22	0·503	6·44	1·46	1·20	0·298	3·48	0·86	10·29	2·566	29·83	7·44
110	XLV	M	76	440	2·90	0·596	6·59	1·355	1·35	0·278	3·07	0·63	14·73	2·988	33·48	6·79
111	XLV	F	76	314	2·00	0·422	6·37	1·344	1·10	0·243	3·51	0·78	9·72	1·916	30·95	6·10
114	XLVI	M	75	370	2·54	0·532	6·87	1·439	1·18	0·231	3·19	0·63	9·10	2·016	24·59	5·45
115	XLVII	M	76	294	2·45	0·512	8·33	1·742	1·10	0·236	3·75	0·80	9·06	1·910	30·82	6·50
117	XLVIII	F	76	342	2·37	0·596	6·93	1·742	1·38	0·352	4·03	1·03	12·10	3·112	35·38	9·10
120	XLIX	F	76	322	2·02	0·420	6·27	1·303	1·08	0·221	3·36	0·69	9·94	1·983	30·87	6·10
123	L	F	76	284	2·38	0·488	8·38	1·718	1·15	0·238	4·05	0·84	10·25	2·110	36·09	7·43
124	L	F	76	300	2·63	0·536	8·77	1·787	1·32	0·264	4·40	0·88	11·38	2·278	37·93	7·60
125	LI	M	76	370	2·58	0·520	6·98	1·406	1·55	0·332	4·19	0·90	15·56	3·168	42·05	8·56
				351			7·09	1·52			3·59	0·78			32·29	6·88
Mean							1·087	0·222			0·421	0·152			4·012	0·995
Standard Deviation							±0·16	±0·03			±0·06	±0·02			±0·59	±0·15
Probable Error of Mean																

Probable Error of Mean						±0·16	±0·03				±0·06	±0·02			±0·59	±0·15

SERIES III.

Excision of Left Kidney.

Group 3.

Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Grammes.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Kidney. Weight. Wet.	Kidney. Weight. Dry.	Kidney. Ratio (× 1,000). Wet.	Kidney. Ratio (× 1,000). Dry.	Heart. Weight. Wet.	Heart. Weight. Dry.	Heart. Ratio (× 1,000). Wet.	Heart. Ratio (× 1,000). Dry.	Liver. Weight. Wet.	Liver. Weight. Dry.	Liver. Ratio (× 1,000). Wet.	Liver. Ratio (× 1,000). Dry.
1	LII	M	76	452	2·95	0·608	6·53	1·34	1·76	0·382	3·90	0·845	20·65	4·388	45·69	9·71
4	LIII	M	76	495	3·25	0·662	6·56	1·34	1·86	0·388	3·76	0·785	18·40	3·728	37·17	7·53
6	LIV	M	76	416	3·02	0·612	7·26	1·47	1·82	0·387	4·38	0·931	14·85	2·989	35·69	7·19
8	LV	M	76	427	2·89	0·591	6·77	1·39	1·68	0·366	3·94	0·858	14·72	2·966	34·47	6·95
10	LVI	M	76	448	2·92	0·591	6·52	1·32	1·70	0·356	3·80	0·795	14·35	2·986	32·03	6·67
12	LVII	F	76	468	2·80	0·566	5·98	1·21	1·82	0·378	3·89	0·807	15·16	3·121	32·39	6·67
14	LVIII	F	76	418	3·00	0·382	7·18	1·46	1·78	0·382	4·26	0·914	16·38	3·318	39·18	7·94
16	LIX	M	76	495	3·62	0·377	7·31	1·46	1·88	0·377	3·80	0·763	24·70	4·988	49·89	10·08
20	LX	F	76	428	3·08	0·786	7·18	1·84	1·74	0·489	4·07	1·142	16·12	4·212	37·67	9·48
21	LXI	F	76	398	2·97	0·755	7·46	1·89	1·86	0·488	4·70	1·226	11·66	2·996	29·29	7·53
23	LXII	F	76	446	3·18	0·796	7·13	1·79	1·89	0·499	4·28	1·118	16·32	4·122	36·59	9·24
26	LXIII	F	76	378	2·60	0·671	6·88	1·78	1·66	0·418	4·39	1·106	15·52	3·902	41·06	10·32
29	LXIV	M	76	410	2·86	0·454	6·98	1·76	1·82	0·450	4·44	1·095	15·73	3·942	38·37	9·62
32	LXV	M	76	496	3·76	0·950	7·58	1·91	1·98	0·498	3·99	1·003	20·06	5·120	40·44	10·32
33	LXVI	F	76	512	3·47	0·876	6·78	1·71	2·12	0·536	4·14	1·045	24·18	6·212	47·23	12·13
36	LXVII	M	76	410	3·46	0·880	8·44	2·15	1·89	0·480	4·61	1·171	15·82	3·980	38·59	9·71
38	LXVIII	F	76	400	3·33	0·838	8·33	2·10	1·72	0·430	4·30	1·075	12·72	3·210	31·80	8·03
40	LXIX	F	76	463	3·66	0·902	7·90	1·95	1·98	0·496	4·28	1·071	19·52	4·662	42·16	10·07
42	LXX	F	52	400	3·28	0·861	8·20	2·15	1·88	0·472	4·70	1·180	18·92	4·718	47·30	11·80
45	LXXI	M	52	365	2·80	0·730	7·67	2·00	1·82	0·420	4·99	1·151	12·68	3·126	34·74	8·56
Mean				436			7·20	1·70			4·23	1·01			38·60	9·00
Standard Deviation							0·663	0·305			0·329	0·158			5·533	1·69
Probable Error of Mean							±0·10	±0·05			±0·05	±0·02			±0·84	±0·26

SERIES III.
Group 3 (continued).

Ligation of Vessels of Left Kidney.

Weight of Organs in Grammes with their Ratios (× 1,000) to Body Weight in Crammes.

Number of Animal.	Number of Litter.	Sex.	Time after Operation.	Body Weight at Death.	Kidney. Weight. Wet.	Kidney. Weight. Dry.	Kidney. Ratio (× 1,000). Wet.	Kidney. Ratio (× 1,000). Dry.	Heart. Weight. Wet.	Heart. Weight. Dry.	Heart. Ratio (× 1,000). Wet.	Heart. Ratio (× 1,000). Dry.	Liver. Weight. Wet.	Liver. Weight. Dry.	Liver. Ratio (× 1,000). Wet.	Liver. Ratio (× 1,000). Dry.
2	LII	F	76	423	2·82	0·571	6·67	1·35	1·42	0·290	3·36	0·69	17·45	3·518	41·25	8·32
3	LIII	M	76	476	3·04	0·612	6·39	1·29	1·90	0·391	3·99	0·82	16·82	3·416	35·34	7·72
5	LIV	M	76	482	3·15	0·646	6·54	1·34	1·79	0·366	3·71	0·76	19·00	3·887	39·42	8·06
7	LIV	M	76	431	3·07	0·622	7·12	1·45	1·72	0·358	3·99	0·83	16·32	3·478	37·87	8·07
9	LV	F	76	416	2·75	0·568	6·61	1·37	1·46	0·299	3·51	0·72	16·42	3·467	39·47	8·33
11	LVI	M	76	432	2·72	0·561	6·29	1·30	1·50	0·316	3·47	0·73	16·05	3·421	37·15	7·92
13	LVII	F	76	415	2·85	0·581	6·87	1·40	1·47	0·299	3·55	0·72	14·28	2·966	34·41	7·15
15	LVIII	F	76	463	3·35	0·678	7·24	1·46	1·67	0·342	3·61	0·74	19·18	3·921	41·49	8·47
17	LIX	M	76	487	3·46	0·698	7·10	1·44	2·01	0·420	4·13	0·86	21·65	4·472	44·45	9·15
18	LIX	F	76	418	2·60	0·671	6·22	1·61	1·76	0·452	4·21	1·08	14·85	3·916	35·53	9·30
19	LX	M	76	465	3·45	0·872	7·42	1·88	2·05	0·521	4·41	1·12	19·15	4·916	41·18	10·57
22	LXI	M	76	459	3·34	0·842	7·28	1·84	2·05	0·526	4·47	1·15	18·78	4·991	40·91	10·87
24	LXII	F	76	427	3·02	0·761	7·07	1·78	1·92	0·488	4·49	1·15	14·59	3·682	34·17	8·62
25	LXIII	F	76	415	3·02	0·761	7·28	1·84	1·88	0·491	4·53	1·18	16·28	4 081	39·23	9·83
27	LXIII	F	76	395	2·87	0·722	7·27	1·83	1·82	0·458	4·62	1·16	16·28	4·081	41·21	10·33
28	LXIV	M	76	400	2·53	0·635	6·33	1·83	1·78	0·445	4·45	1·16	13·82	3·456	34·55	8·97
30	LXV	F	76	403	3·06	0·766	7·59	1·90	1·86	0·466	4·62	1·16	16·40	4·202	40·69	10·42
34	LXVI	M	76	487	3·12	0·782	6·41	1·60	2·02	0·516	4·19	1·06	16·55	4·162	33·98	8·55
35	LXVII	M	76	456	3·38	0·822	7·71	1·80	1·98	0·472	4·34	1·06	22·00	5·612	48·24	12·32
41	LXIX	F	76	498	3·65	0·920	7·33	1·85	2·12	0·530	4·26	1·06	26·12	6·700	52·45	13·45
44	LXXI	F	52	380	2·88	0·720	7·58	1·90	1·68	0·420	4·42	1·11	11·72	2·812	30·84	7·40
				439											39·14	9·21
Mean							6·96	1·62			4.12	0.97			5·676	1·55
Standard Deviation							0·491	0·226			0·430	0·176				
Probable Error of Mean							±0·07	±0·03			±0·06	±0·03			±0·84	±0·23

THE EFFECT OF TEMPERATURE ON THE UNEQUAL INTAKE OF THE IONS OF SALTS BY PLANTS

by

ARTHUR K. H. PETRIE

(From the Department of Botany, University of Melbourne).

(Submitted for publication 12th July, 1927.)

"If Donnan's theory of membrane equilibria furnishes the mathematical and quantitative basis for a theory of colloidal behaviour of the proteins, as the writer believes it does, it may be predicted that this theory will become one of the foundations on which modern physiology will have to rest."—Loeb (24).

INTRODUCTION.

It has been known for some considerable time that, when a plant tissue or root-system is immersed in a solution of an electrolyte, the two ions of the electrolyte are absorbed in different proportions, and in the case of neither ion does absorption necessarily proceed to an equilibrium position characterized by equality of concentration within and without. For this conclusion, indeed, there is now an accumulation of evidence: Meurer (28), Ruhland (42), and in particular Stiles (44), found that the ions of salts were absorbed in unequal proportions by isolated storage tissues; and Pantanelli (34), Redfern (37), and Hoagland (20) observed the same phenomenon in the case of absorption by entire plants. Unequal internal and external equilibrium concentrations of ions have also been found in the case of *Nitella* by Hoagland and Davis (21), and by Osterhout (32) in the case of *Valonia*. Not without interest in this connection, too, is the heaping up of the coloured ion of certain dyes in plant cells, first observed by Pfeffer (36), and the comparatively small absorption in the case of others (Collander, 8). Many other observations of a like nature could be quoted, details of some of which may be obtained from the review of Stiles (43).

These results point to the existence of some physico-chemical mechanism controlling absorption, whereby some ions are trapped and accumulated in the protoplast and others are largely excluded: a mechanism of this kind must be very fundamental in the life-processes of the cell, and it is likely to be intimately associated with many phenomena, such as antagonism, selective absorption from

the soil, and, indeed, all the manifold interrelationships between the plant and its nutrient medium.

Of the nature of this mechanism we have much to learn; for in all the above-quoted work there is no suggestion of a definite rule operating in the absorption processes; in some cases the anion was absorbed in excess, in other cases the cation. It has been shown by Stiles (44), however, that the equilibrium position in the intake of an ion is dependent on its concentration: with decreasing external concentration relatively more salt was found to be absorbed, the equilibrium being given approximately by the equation:

$$y = kc^m,$$

where y is the final internal concentration, c the final external concentration, and k and m are constants. This equation is that which has been held to be characteristic of adsorption processes, and has consequently led Stiles to believe that adsorption may be a governing force in the absorption of the ions of salts by plant cells.

Apart from this, however, practically no investigation has been made of the influence of external factors on the equilibrium position in the absorption of ions by plants; and the present paper has been devoted to the study of the influence of temperature on the equilibria of ionic intake, in the hope of making a further contribution to our knowledge of the underlying mechanism of this process. The temperature factor was specially selected for study, since it has received perhaps least previous consideration; our only knowledge of its effect being a few observations by Miss Redfern (38), which indicated that, in the intake of methylene blue by carrot tissue, absorption decreased with rise in temperature. No investigation has been made of the effect of this factor on the unequal intake of the two ions of a single electrolyte, to which attention has been particularly directed in the present work.

It is a pleasure to record my gratitude to Professor A. J. Ewart, F.R.S., who has done much to facilitate the performance of this investigation; I am indebted also to Professor T. Brailsford Robertson, of the University of Adelaide, for kind suggestions and criticism. The expenses of the experimental work were defrayed in part by a grant from the research funds of the University.

EXPERIMENTAL.

Technique.

The method adopted in studying absorption in the present investigation was that which has been brought into use by Stiles (44). The parenchymatous tissue from the central region of the root of the carrot was used in the form of circular discs 1 mm. in thickness and 17·5 mm. in diameter. These discs were prepared by means of a cork-borer and a sliding microtome, and were then washed for twelve hours in running tap-water, followed by successive changes of distilled

water extending over several hours. This process was carried out in order to remove material from the cut surface cells and also as much as possible such substances as might tend at first to diffuse out from the tissue; it served also to allow the tissue to attain a uniform and maximum degree of turgidity.

After washing, the discs were drained on filter paper in sets of forty, each set then being placed in a flask containing 100 cc. of the solution of which the absorption was to be measured. In any series of solutions used in an experiment, each set of discs was taken from the same original batch of carrots, as it was found that different batches showed a certain amount of variation in the results given, although the main conclusions to be drawn were not modified. In order to ensure that the tissue remained healthy, Stiles weighed the sets of discs before and after the experiment, and concluded by the constancy of the weight whether turgidity had been maintained. It was found, however, that death of the tissue, resulting in complete loss of turgor, could readily be observed by the flabby nature of the discs in such condition, so that the weighing was dispensed with in later experiments; and, moreover, loss in weight, owing to osmotic effects resulting from immersion in the experimental solution, may occur without affecting the vitality of the cells.

The process of absorption was followed by removing samples at intervals and analyzing them, until it was seen by the flattening of the curve of absorption in relation to time that equilibrium had been reached. On the removal of each sample, discs were taken out in such number as to keep constant the ratio of the number of discs to the volume of the liquid. In the tables of results only the equilibrium values for absorption have been given.

It was found that equilibrium was generally attained when about eighty hours had elapsed from the commencement of the experiment. This was a much longer period than was required in the case of Stiles' experiments, but in his case the preparations were agitated continuously during the process of absorption, while in the present instance mixing could be effected only at intervals of some hours.

A number of sets of discs immersed in the experimental solution were prepared for each experiment, and these were kept at various temperatures, ranging from 4–30°C. These temperatures were obtained in a special laboratory artificially kept at 10°C. Temperatures above this value were maintained in ovens controlled by gas-mercury-toluene thermostats, and lower temperatures were obtained in a refrigerator, the unvarying temperature of the room rendering all values constant to within 1°C.

Difficulty was experienced at first in keeping the samples above 20°C. free from infection, and for this reason sterilization of the laboratory and all apparatus and solutions became necessary. It was found also that above 30° the tissue tended to die before the equilibrium position was reached, the discs losing turgor and becoming quite flabby.

The hydrogen-ion concentration of the various solutions was determined at the beginning and end of most of the experiments, to ensure that changing reaction might not be in part responsible for the results obtained. These estimations were carried out by the indicator method, using standard buffer solutions for comparison.

Experiments were conducted, using the dyes methylene blue and erythrosin A in ·005% aqueous solution, and the salts ammonium chloride, ammonium sulphate, potassium chloride, and calcium nitrate in ·1M. concentration.

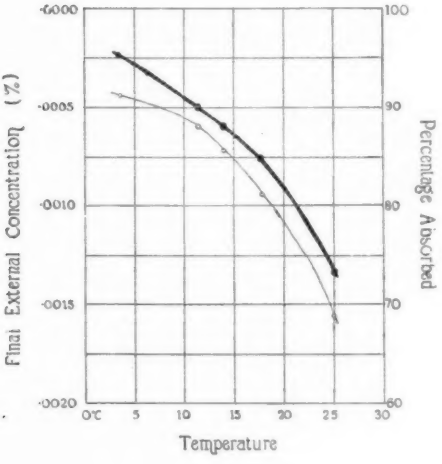


Figure 1. Curves showing the relation of the final external concentration to temperature in the absorption of ·005% methylene blue in two typical experiments. The upper curve is constructed from the results given in Table 1.

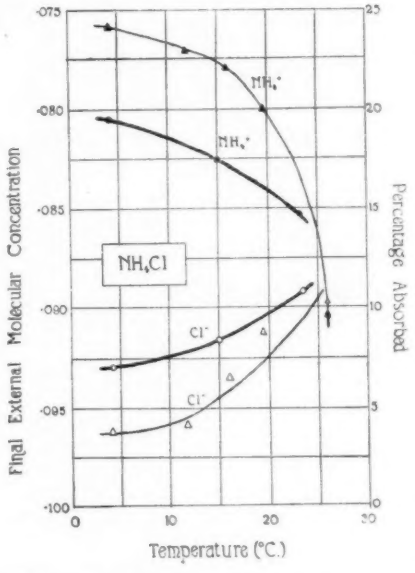


Figure 2. Curves showing the relation of the final external concentration of NH_4^+ and Cl^- to temperature in the absorption of ·1M ammonium chloride in two typical experiments. The heavy curves are constructed from the results in Table 2.

TABLE 1.

Equilibrium Position in the Intake of ·005% Methylene Blue at Various Temperatures.

Temperature.	Initial Ext. Concn.	Final Ext. Concn.
25°C.	·0050%	·00133%
18	,,	·00074
14	,,	·00058
11	,,	·00050
3	,,	·00023

Results.

The Intake of Methylene Blue.

The investigation was commenced with a study of the absorption of methylene blue, in order to compare the results obtained with those of Redfern (38) previously mentioned. The absorption was measured in ·005% aqueous solution of the dye, at temperatures of 25°, 18°, 14°, 11°, and 3°C., the methylene blue being estimated colorimetrically. In each case the experiment was repeated a number of times with remarkably uniform results, which as a whole exhibited the same trend as those of Redfern, although they differed numerically to a considerable extent. In Table 1 are given the results of a typical experiment with methylene blue, these being also expressed graphically in figure 1.

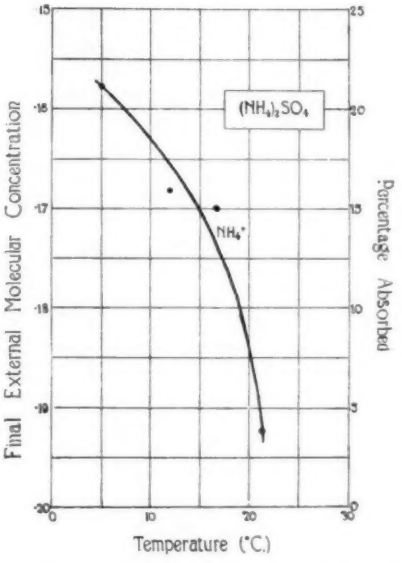


Figure 3. Curve showing the relation of the final external concentration of NH_4^+ to temperature in the absorption of ·1M $(NH_4)_2SO_4$. Constructed from results given in Table 3.

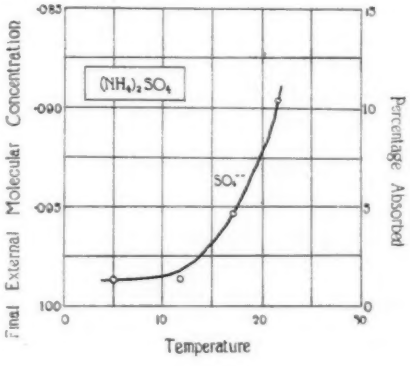


Figure 4. Curve showing the relation of the final external concentration of SO_4'' to temperature in the absorption of ·1M $(NH_4)_2SO_4$. Constructed from results given in Table 3.

The Intake of Erythrosin.

Experiments were conducted in a precisely similar manner to those with methylene blue, using a ·005% aqueous solution of erythrosin A. The amount of absorption, however, was small, and no marked temperature effect was

obtained; moreover, accurate estimation was rendered difficult on account of changes which took place in the colour of the dye during the course of the experiment. For these reasons the use of erythrosin was discontinued, more especially as the main interest of the investigation lay in the use of salts, of which both ions could be readily estimated, rather than in the use of dyes.

The Intake of Ammonium Chloride.

The absorption of ·1M ammonium chloride was observed under conditions similar to those of the previous experiments on dyes. Ammonium was estimated colorimetrically after Nesslerization, while chlorine was estimated by the process of Volhardt. In Table 2 are given the results of a typical experiment. The relation of the final external concentration to temperature is also expressed graphically in figure 2.

The Intake of Ammonium Sulphate.

The previous experiments were repeated, using ·1M ammonium sulphate. Ammonium was estimated as before, and the sulphate radicle was precipitated and weighed as barium sulphate. Typical results are given in Table 3 and figures 3 and 4.

TABLE 2.

Equilibrium Position in the Intake of the Ions of ·1M NH_4Cl *at Various Temperatures.*

	NH_4'		Cl'				
Temp.	Init. ext. concn.	Final ext. concn.	Init. ext. concn.	Final ext. concn.	$NH_4' \times Cl'$	Original P_H of solution.	Final P_H of solution.
4°C.	·100M	·0805M	·100M	·0930M	·00748	6·7	5·8
15	,,	·0825	,,	·0916	·00755	6·7	5·7
23	,,	·0855	,,	·0892	·00758	6·7	6·0

The Intake of Potassium Chloride.

The absorption of ·1M potassium chloride was studied in a manner similar to that used for the previous salts. Potassium was estimated by precipitating with sodium cobaltinitrite (Macallum's reagent, 25), according to a method similar to that originally devised by Aidie and Wood (1). Five cubic centimetre samples of the experimental solution were used, and to each of these 10 cc. of the reagent were added. After standing for twelve hours the precipitate was filtered in tared filters, washed, and dried at 125°C. to constant weight. As a microchemical process the accuracy of this method has been subjected to

some criticism, but with the large quantity of precipitate used in the present estimations satisfactory results were readily obtained. In carrying out these analyses I received much assistance from my father, the late Dr. J. M. Petrie, of the University of Sydney, who had made this method a subject of special study. The chlorine, as in previous cases, was estimated by Volhardt's method. Results of a typical experiment are give in Table 4 and figure 5.

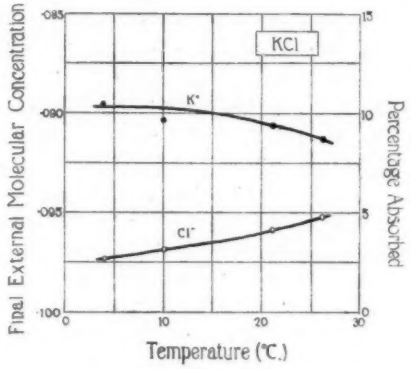


Figure 5. Curves showing the relation of the final external concentration of $K^{\cdot}$ and Cl' to temperature in the absorption of ·1M potassium chloride. Constructed from results given in Table 4.

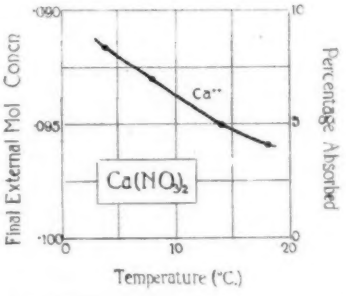


Figure 6. Curve showing the relation of the final external concentration of $Ca^{\cdot\cdot}$ to temperature in the absorption of ·1M Ca $(NO_3)_2$. Constructed from results given in Table 5.

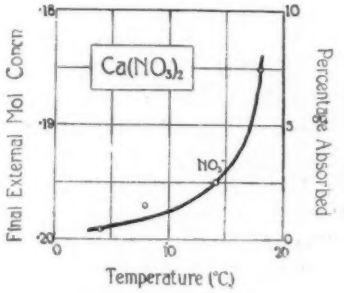


Figure 7. Curve showing the relation of the final external concentration of NO_3' to temperature in the absorption of $Ca(NO_3)_2$. Constructed from results given in Table 5.

The Intake of Calcium Nitrate.

Similar experiments were carried out on the absorption of ·1M calcium nitrate. Calcium was estimated by precipitating with oxalic acid and titrating

the excess acid, while the nitrate was estimated colorimetrically after conversion to ammonium picrate. The results of a typical experiment are given in Table 5 and figures 6 and 7.

DISCUSSION.

The Results Analysed.

The results of the experiments carried out with methylene blue indicate that with increasing temperature there is a progressive fall in the equilibrium intake of the dye. The same tendency was shown in the few observations made on this subject by Miss Redfern, as has already been mentioned.

The curves showing the relation of the final external concentration to temperature (figure 1) indicate that, especially at the lower temperatures, a very large proportion of the dye is absorbed into the tissue. In this connection it is interesting to bear in mind that methylene blue is a basic dye, or in other words, the results obtained pertain to the absorption of the positive ion only; and, while this accumulation within the tissue characterizes the basic dye, the absorption of the acid dye, erythrosin, or rather its acid radicle, which is the coloured portion, was only slight. These facts are in agreement with Pfeffer's observations (36), more recently confirmed by Redfern (38)[1], that while plant cells rapidly absorb basic dyes, acid dyes are in general absorbed only to a slight extent or not at all. They are also in agreement with Ruhland's earlier adherence to this view (40, 41), and with Overton's conclusion that only dyes soluble in lipoid substances enter the cell (33), since on the whole basic dyes are soluble in lipoids, while acid dyes are not.

These experiments with dyes, however, are of a subsidiary nature: the main interest of the investigation lies in the results obtained with such electrolytes as ammonium and potassium chlorides, ammonium sulphate and calcium nitrate, wherein both cation and anion were estimated. If the curves in the various figures are examined, it will be seen that a single general principle is operating in them all, independent of the particular salt used. It is moreover apparent that the effect of temperature on the equilibrium concentration is remarkably different for the two ions of the salt: as with methylene blue, the equilibrium intake of the cation falls as the temperature rises; but, on the other hand, the amount of the anion absorbed increases with the temperature. From the variety of salts used, it would appear that these are fundamental properties to be associated with monovalent and divalent cations and anions in general; and it will be the purpose of the remaining pages to consider how these properties may be interpreted.

---

[1]Miss Redfern in this paper falls into the error of supposing that aniline blue is a basic dye; in reality it is acidic, and it is noteworthy that Miss Redfern finds that it behaves in a manner similar to the other acid dyes which she used.

TABLE 3.

Equilibrium Position in the Intake of the Ions of ·1M $(NH_4)_2SO_4$ *at Various Temperatures.*

	NH_4'		SO_4''		
Temp.	Init. ext. concn.	Final ext. concn.	Init. ext. concn.	Final ext. concn.	$(NH_4')^2 \times (SO_4'')$
5°C.	·200M	·158M	·100M	·0985M	·0033
12	,,	·167	,,	·0985	·0028
17	,,	·170	,,	·0953	·0028
21·5	,,	·192	,,	·0897	·0025

THE SIGNIFICANCE OF THE ABSORPTION FORMULA.

In the Introduction to this paper it was mentioned that in the absorption of an ion Stiles had found the equilibrium position to be dependant on the concentration, in accordance with the equation

$$y = kc^m.$$

This equation was found by Redfern (38) to hold also in the case of the absorption of the coloured radicles of dyes by carrot tissue.

The importance of Stiles' discovery is that this equation is identical with the adsorption isotherm of Freundlich (16), which connects the amount of the so-called process of "adsorption" with the equilibrium concentration. This equation has been held by many authorities in the past to be characteristic of the physical process of surface aggregation as contrasted with actual chemical combination; and, in the light of the above results, as was mentioned in the Introduction, Stiles has suggested that the ions taken in by the tissue are "adsorbed" at the surface of some cell constituent or constituents, probably colloidal in nature. In support of this view some authorities might cite also the few observations of Redfern indicating a decrease in the percentage absorption of dyes with increase of temperature, a property likewise held to be "characteristic of adsorption processes as distinct from chemical combinations" (38) (see also Bayliss, 4, p. 61). It may be remarked here, however, that the results of the present investigation, while similar to those of Redfern in the case of methylene blue and cations in general, are not so in the case of anions, and, if a negative temperature coefficient is held to be a characteristic property of adsorption, then this process is not a direct cause for the intake of these ions.

At the same time Stiles has pointed out that chemical combination of the absorbed salt with a cell constituent is unlikely, since in such terms it would not be possible to explain the fact, manifested in his results, that the ratio of the final internal to the final external equilibrium concentration, which he terms

the absorption ratio, is less than unity in the case of the higher concentrations of the solution. "For," as he states, "if the salt is capable of combining with a cell constituent, it must be concluded that it is capable of entering the cell, and it would then be supposed that, after all the particular cell constituent combining with the salt was utilized, absorption of the salt would continue until there was equality of concentration of the salt on the two sides of the cell-membranes. The chemical combination view, therefore, also involves the assumption that the salt itself, or the compound formed by its action within the cell, renders the cell membranes impermeable to the salt, so that absorption stops before enough of the salt is taken up to bring about equality of concentration on the two sides of the membrane. The very regular relation between concentration and absorption, however, militates against the view that a cell-membrane is rendered impermeable in high concentrations of the salt, while low concentrations have no such action."

The chemical combination view, however, does not necessarily involve such assumptions as Stiles would suppose, for it has been shown that many purely chemical reactions in a heterogeneous medium conform to an equation of the Freundlich type, a frequently quoted example being the union between haemoglobin and oxygen. Michaelis and Rona (31) have also shown that the absorption of basic dyes by filter paper is not a process of physical adsorption, as was formerly supposed (Bayliss, 4), but is due to a chemical substitution of the basic radicle of silicates in the paper by the base of the dye. Loeb (24) has shown that adsorption by proteins is a stoichiometric replacement of hydrogen or basic ions according to the state of the protein with reference to its isoelectric point. Fisher (15) in studying the phenomena of absorption in soils has demonstrated that the Freundlich equation applies with an equal degree of accuracy to many reactions that are not adsorptions, and he points out also that, as the exponent m is altered, a series of curves is obtained to one of which nearly any set of experimental points can be fitted with the desired degree of accuracy: adsorption in soils is to be explained as a base exchange in the salts of the weak colloidal acids which are now known to be among their most important constituents (5, 6, 19). The Wilsons (47) have rejected the adsorption formula as applying to colloidal problems, and have adopted a purely chemical interpretation; and its unsoundness as a criterion of any particular type of reaction has been urged by many other writers, e.g., Arrhenius (2), Bradfield (5), Gordon (17), Langmuir (22), Robertson (39). There seems every reason to believe, as Mathews (27) has suggested, that adsorption of most substances from solution, including acids, bases, ions, salts, dyes, etc., is due to chemical forces, the adsorbed substance forming a true chemical union with the surface molecules of the adsorbent.

TABLE 4.

Equilibrium Position in the Intake of the Ions of ·1M KCl *at Various Temperatures.*

Temp.	$K^{\cdot}$ Init. ext. concn.	$K^{\cdot}$ Final ext. concn.	$Cl^{\prime}$ Init. ext. concn.	$Cl^{\prime}$ Final ext. concn.	$K^{\cdot} \times Cl^{\prime}$	Original P_H of solution.	Final P_H of solution.
4°C.	·100M	·0896M	·100M	·0973M	·0087	6·6	5·8
10	,,	·0904	,,	·0969	·0088	6·6	6·0
21	,,	·0906	,,	·0958	·0087	6·6	5·8
26	,,	·0912	,,	·0953	·0087	6·6	5·8

It appears, then, unnecessary to suppose, with Stiles, that the chemical combination theory is incompatible with the adsorption equation: in a process of base exchange, for example, the extent to which replacement takes place must vary exponentially with the equilibrium concentration of the entering cation, and so conform with the general types of Freundlich's equation, although at the same time the replacement is a chemical one. And judging by analogy with the works that have been quoted, a chemical interpretation for the absorption of ions by plant cells is perhaps more likely than one relying upon purely physical phenomena. We have digressed thus far to arrive at this conclusion, since it is in terms of a chemical combination theory that a possible interpretation of the present results seems to lie.

BASE EXCHANGE IN COLLOIDAL ACIDS.

From the results of various investigators it seems well established that, in the unequal absorption of the constituent ions of a salt, the excess of cation over anion absorbed is replaced in the external solution by the diffusion out from the tissue of an equivalent amount of other positively charged ions. Thus Stiles (44) has shown that in the absorption of sodium chloride by carrot tissue the excess $Na^{\cdot}$ absorbed is replaced by $Ca^{\cdot\cdot}$, $K^{\cdot}$ and $Mg^{\cdot\cdot}$; Redfern (37) likewise found that in the absorption of calcium chloride by living plants, the excess of $Ca^{\cdot\cdot}$ ions was replaced by $Mg^{\cdot\cdot}$ and $K^{\cdot}$; and Stocklasa and his collaborators (45) found a similar replacement by $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$ and $Na^{\cdot}$ in the case of the absorption of $Al^{\cdot\cdot\cdot}$ and $Fe^{\cdot\cdot\cdot}$ ions.

These results leave no doubt that the process of cation absorption is one of base exchange. Now in our discussion of adsorption, it has been realized that many reactions characterized by the adsorption formula are in reality cases of chemical exchange of bases in colloidal salts; and one is thus led to consider the possibility of a similar interpretation for the phenomenon at present under consideration. Such a conception would be reconciled with Stiles' results

indicating the régime of the adsorption formula; and, moreover, in the light of our previous conclusions upon the significance of this formula, its very applicability to electrolytic absorption by plant tissue points, if to anything, to a colloidal base exchange such as is here suggested.

The existence of such a negative colloidal radiele as would operate in a process of base exchange of this kind is no assumption. It is well known that protoplasm contains many colloids, numbers of which must be in the form of negative ions. Among these are obviously the proteins: the chief protein in carrot tissue is a globulin of which the isoelectric point is at P_H 4·3 (7); and, since the normal reaction of the tissue is about P_H 6·0 (35) and the final external P_H in the experiments described in the present paper was never as low as 4·3, the protein in the cell-protoplasm must exist in the form of colloidal anions, with which base exchange could readily take place. One may mention also the phosphatides which, as Levene (23) has pointed out, are amphoteric like the proteins, and may play a fundamental rôle in the metabolic processes of the cell (cf. 9, 18). Perhaps of more significance, however, on account of their greater profusion in plant protoplasm (cf. Macdougal, 26), are the carbohydrates: many of these substances are known to be dissociated as weak acids or their salts (Euler, 12, 13; Michaelis and Rona, 29, 30) and it seems highly probable that the colloidal polysaccharides occurring in plant protoplasm will behave in the same way; Fairbrother and Mastin (14) have, in fact, shown that this is so in the case of sugar, and have also shown that base exchange with this colloidal acid salt takes place with ease. We have not yet the evidence to suggest which of these substances might be concerned in the present problem; but it is clear that colloidal anions, with which replaceable basic ions must be associated, occur in abundance in plant protoplasm.

TABLE 5.

Equilibrium Position in the Intake of the Ions of ·1M $Ca(NO_3)_2$ *at Various Temperatures.*

Temp.	$Ca^{\cdot\cdot}$ Init. ext. concn.	$Ca^{\cdot\cdot}$ Final ext. concn.	NO_3' Init. ext. concn.	NO_3' Final ext. concn.	$Ca^{\cdot\cdot} \times (NO_3')^2$	Original P_H of solution.	Final P_H of solution.
4°C.	·100M	·0917M	·200M	·199M	·0036	10·0	6·6
8	,,	·0930	,,	·197	·0036	10·0	6·6
14	,,	·0950	,,	·195	·0036	10·0	6·4
18	,,	·0959	,,	·185	·0033	10·0	6·4

The Donnan Equilibrium.

The system as we conceive it in the plant cell may now be regarded fundamentally as one of two solutions separated by a semi-permeable membrane. The internal solution is that of the colloidal salts in the protoplasm, separated from the external experimental solution by the plasma-membrane of the cell. To all the crystalloidal ions in the system this membrane is to be regarded as permeable, while on the contrary it is impermeable to the colloidal anions within.

A system such as this is one in which we might well suppose that a Donnan equilibrium would operate. It has been shown by Donnan (10, 11) that, when two solutions of electrolytes are separated by a membrane which is impermeable to one of the ions of one of the electrolytes, the diffusible ions proceed to an equilibrium at which they are unequally distributed on the two sides of the membrane. At this equilibrium, also, the product of the concentrations of each pair of oppositely charged ions is the same on either side; and these products are constant whether only a single electrolyte is concerned or whether there is a mixture of a number.

This equilibrium condition can be expressed by the equation

$$x^2 = y(y + z),$$

where x is the concentration of the positive and of the negative ions on one side of the membrane, y is the concentration of the positive and of the negative free crystalloidal ions on the side containing the indiffusible ion, and z is the concentration of crystalloidal ions in combination with the indiffusible ion.

The membrane equilibrium can be expressed also by the equation

$$ax = iy,$$

where a and x are the concentrations of any pair of oppositely charged ions in the one solution, and i and y are the total concentrations of the same pair of ions on the other side of the membrane.

If one of the ions, e.g. the negative one (x,y) is divalent, these equations become

$$x^3 = y^2(y + z)$$

and

$$a^2x = i^2y.$$

It is no new departure to suggest the application of the Donnan equilibrium to living protoplasm: Donnan himself stressed the importance of its potential biological applications on account of the existence in living organisms of just such semi-permeable membranes as the theory requires. This has been further upheld by Loeb (24); and more recently several investigators have applied the Donnan theory to the distribution of electrolytes in blood (3, 46, 48). It will be the purpose of the concluding pages to show that, if we assume this theory to hold in the case of plant cells also, it provides an interpretation for the results obtained in the present inquiry.

The Results Interpreted.

In the case of the non-diffusible ion being negative, as has been concluded to be the case in the present instance, then of any pair of crystalloidal ions in the system, the Donnan theory requires that the external concentration of the cation should be less than the internal, and that the reverse should be the case with the anion. Starting then with equal concentrations of ions outside, when the equilibrium position has been reached the external concentration of cation should be less than that of the anion, while the reverse should hold for the internal concentrations: stated more simply, i will be greater than y, and a will be less than x.

It will be observed that this relationship of a to x holds in all the experiments recorded in the present work: and it is obvious that the relationship of i and y must hold also, since the internal concentrations, although not capable of estimation experimentally, must be in inverse ratio to the external concentrations.

The results show, however, that with increase of temperature the value of a rises and that of x falls, wherefore the excess of i over y must decrease. Such a decrease would be explained if the effect of rising temperature were such as to cause a decrease in the value of z, the concentration of the indiffusible negative ion.

Now such a decrease is what might well be expected to follow rise in temperature. Many colloidal suspensions, e.g. powdered gelatine, tend to pass into true solution under such conditions; and, passing into solution, might then be able to diffuse out through the plasma-membrane and so become less concentrated within the cell: or their exosmosis might occur at higher temperatures purely as a result of the increasing permeability of the plasma-membrane, which is known to be proportional to the temperature. In the case of at least one of the colloidal anions of the protoplasm, viz., the phosphatides, it is definitely known that outward diffusion occurs in increasing amount with elevation of temperature (9).

In any case, as Professor T. Brailsford Robertson, of the University of Adelaide, was kind enough to point out to me, it may be taken as a general rule that rising temperature decreases the combining capacity of tissue colloids for bases, which would be equivalent to a decrease in the value of z. I am indebted to Professor Robertson also for the suggestion that polymerization of the colloid with higher temperatures, as for example is known to take place on coagulation, might reduce its combining capacity for bases, and so reduce the concentration of colloidal anions.

If, then, we are justified in suggesting that the value of z falls with rising temperature, it follows from the Donnan equations that, since the total amount of the experimental electrolyte in the system does not alter, not only should ax equal iy at any particular temperature, but the values of these products

must remain the same at all temperatures; and the same should hold for the two sides of the equation as modified for difference in the valency of either of the ions.

It is unfortunately impracticable to determine experimentally whether the external and internal ionic products are equal, on account of the impossibility of determining the internal concentrations of the ions. Stiles (44), it is true, has calculated empirical values for these concentrations, based on the difference between the initial and final concentrations. Values obtained in this way, however, would have no significance in the present case, since Stiles has to assume an empirical and constant estimate for the volume of the solvent containing the electrolyte in the tissue. It is thus obvious that Stiles' calculations of the internal concentration are not only empirical but may not even be proportionally correct, since the volume of internal solvent is certain to change from the assumed constant value with alteration of temperature.

So far as the external concentrations are concerned, however, the results of this investigation have shown that their products in any given experiment have a constant value independent of the temperature, which, as we have seen, is what the Donnan theory requires.

On the whole there seems much in support of an interpretation of the absorption of the ions of electrolytes by plants as a process of base exchange in colloidal acids or their salts, such process being governed by the Donnan equilibrium: this conception has at all events provided an interpretation for the results of this inquiry which does not seem to be readily forthcoming in other terms.

It might be urged, however, that since we are adopting a theory based on chemical foundations, it should be possible to demonstrate that stoichiometric relationships exist, and that only the valency of the ions concerned should produce variations in the curves obtained; whereas not only do the curves differ somewhat for various salts, but even for the absorption of the same salt by different batches of tissue. Such stoichiometric relationships, however, could be demonstrated only by the use of material in a precisely similar state for each experiment, which in living organisms it is impossible to obtain. Every batch of tissue must contain somewhat different amounts of colloids, and inevitable fluctuations in the internal hydrogen-ion concentration must cause differences in their degree of dissociation, and hence a further alteration in the value of z in the Donnan equation; except in the case of the proteins, moreover, the degree of dissociation of the colloids may alter with different bases[2]; and variations in the previous history of the tissue may lead to dissimilar concentrations of the ions to be absorbed already being present in the tissue, which again would result in absorption proceeding to a different extent,

[2]Non-ionized molecules can be considered as if wholly removed by precipitation; it is particularly when partial precipitation takes place that Fisher (15) has shown the adsorption formula to apply to chemical combinations.

and would further help to explain inaccuracies in the empirical determinations of the internal concentrations. Only the relative shapes of the curves and the constancy of the external products can be regarded as unfailing criteria under all conditions of the material; and to these criteria the results of the various experiments hold true.

It is not the purpose of this paper to put forward the Donnan equilibrium and stoichiometric base exchange as being the controlling factors in the unequal intake of the ions of electrolytes by living plants. It merely suggests that, if these phenomena indeed take place, as is not impossible, they will provide a satisfactory interpretation of our present knowledge of this process of ionic intake which no other theory seems to give; should it ultimately be possible by deeper and more extensive researches to establish the régime of these factors as unquestioned, then it is perhaps no rash assumption to suggest that its significance will equal that of any former discovery in the nutrient relationships of the living cell.

SUMMARY.

The experimental portion of this paper comprises a study of the effect of temperature on the absorption of the ions of single electrolytes by isolated plant tissue.

It is found that with increasing temperature the amount of cation absorbed falls and the amount of anion absorbed increases; and that these alterations take place proportionally, resulting in a constancy of the products of the two ions in the external solution.

Reasons are given for the probability that the absorption of ions by plant cells is a process of base exchange in dissociated colloidal acids or their salts; and it is suggested that a Donnan equilibrium may be set up between the colloidal anions of the protoplasm on the one hand and the external medium on the other.

Such an hypothesis provides a satisfactory explanation for the results obtained on the effect of temperature on the unequal absorption of the ions of electrolytes, an explanation which does not seem to offer itself in terms of any other theory.

REFERENCES.

1. Adie, R. H. and Wood, T. B.: Journ. Chem. Soc., 77 (1900), p. 1076.
2. Arrhenius, S.: Meddel. k. Vetenskapsakad. Nobelinstitut., 2 (1911), No. 7.
3. Barcroft, J., Bock, A. V., Hill, A. V., Parsons, T. R., Parsons, W. and Shoji, R.: Journ. Physiol., 56 (1922), p. 157.
4. Bayliss, W. M.: Principles of General Physiology, 4th ed., London (1924).
5. Bradfield, R.: Journ. Phys. Chem., 28 (1924), p. 170.

6. Bradfield, R.: Soil Science, 17 (1924), p. 411.
7. Cohn, E. J., Gross, J. and Johnson, O. C.: Journ. Gen. Physiol., 2 (1919), p. 145.
8. Collander, R.: Jahrb. f. wiss. Bot., 60 (1921), p. 354.
9. Cranner, B. H.: Planta, oder Archiv f. wiss. Bot., 2 (1926), p. 438.
10. Donnan, F. G.: Z. Elektrochem., 17 (1911), p. 572.
11. Donnan, F. G.: Chem. Revs., 1 (1924), p. 73.
12. Euler, H.: Ber. d. Chem. Ges., 39 (1906), p. 344.
13. Euler, H. und Beth af Ugglas: Z. Phys. Chem., 68 (1909), p. 502.
14. Fairbrother, F. and Mastin, H.: Journ. Chem. Soc., 123 (1920), p. 1412.
15. Fisher, E. A.: Trans. Farad. Soc., 17 (1922), p. 305.
16. Freundlich, H.: Kapillarchemie. 2te Aufl., Leipzig (1922).
17. Gordon, N. E.: Science (N.S.), 58 (1923), p. 495.
18. Grafe, V. and Magistris, H.: Planta, oder Archiv f. wiss. Bot., 2 (1926), p. 429.
19. Hissink, D. J.: Trans. Farad. Soc., 20 (1924), p. 551.
20. Hoagland, D. R.: Science (N.S.), 48 (1918), p. 422.
21. Hoagland, D. R. and Davis, A. R.: Journ. Gen. Physiol., 5 (1923), p. 646.
22. Langmuir, I.: Journ. Am. Chem. Soc., 40 (1918), p. 1361.
23. Levene, P. A.: Physiol. Revs., 1 (1921), p. 327.
24. Loeb, J.: Proteins and the Theory of Colloidal Behaviour, 2nd edition, New York (1924).
25. Macallum, A. B.: Journ. Physiol., 32 (1905), p. 95.
26. MacDougal, D. T.: Carneg. Inst. Wash. Publ., 297 (1920).
27. Mathews, A. P.: Physiol. Revs., 1 (1921), p. 553.
28. Meurer, R.: Jahrb. f. wiss. Bot., 46 (1909), p. 503.
29. Michaelis, L. and Rona, P.: Biochem. Z., 49 (1913), p. 23.
30. Michaelis, L.: Biochem. Z., 65 (1914), p. 360.
31. Michaelis, L. and Rona, P.: Biochem. Z., 103 (1920), p. 19.
32. Osterhout, W. J. V.: Journ. Gen. Physiol., 5 (1923), p. 225.
33. Overton, E.: Jahrb. f. wiss. Bot., 34 (1900), p. 669.
34. Pantanelli, E.: Jahrb. f. wiss. Bot., 56 (1915), p. 689.
35. Pearsall, W. H. and Ewing, J.: New Phyt., 23 (1924), p. 193.
36. Pfeffer, W.: Untersuch. aus den bot. Inst. zu Tübingen, 2 (1886), p. 179.
37. Redfern, G. M.: Ann. Bot., 36 (1922), p. 167.
38. Redfern, G. M.: Ann. Bot., 36 (1922), p. 511.
39. Robertson, T. B.: Koll. Z., 3 (1908), p. 49.
40. Ruhland, W.: Ber. d. bot. Ges., 26a (1908), p. 772.
41. Ruhland, W.: Jahrb. f. wiss. Bot., 46 (1908), p. 1.
42. Ruhland, W.: Z. f. Bot., 1 (1909), p. 747.
43. Stiles, W.: New Phyt., Reprint No. 13 (1924).

44. Stiles, W.: Ann. Bot., 38 (1924), p. 617.
45. Stocklasa, J. unter Mitwirkung von Sebor, J., Tymich, F. und Swacha, J.: Biochem. Z., 128 (1922), p. 35.
46. Van Slyke, D. D., Wu, H. and McLean, F. C.: Journ. Biol. Chem., 56 (1923), p. 765.
47. Wilson, J. A. and Wilson, W. H.: Journ. Am. Chem. Soc., 40 (1918), p. 886.
48. Warburg, E. J.: Biochem. Journ., 16 (1923), p. 153.

ON THE ORIENTATION OF MITOCHONDRIA IN THE SURFACE CYTOPLASM OF INFUSORIANS

by

E. S. HORNING

(From the Department of Zoology, University of Melbourne).

(Submitted for publication 12th July, 1927.)

INTRODUCTION.

It will be noticed, when surface sections of infusorians are carefully examined, following a mitochondrial technique, that the mitochondria present a striking orientation at the surface of these organisms. This interesting orientation has been observed by several authors, and the object of this paper is to investigate and suggest an interpretation for this impressive phenomenon.

MATERIAL AND METHODS.

In order to carry out this investigation two common types of infusorians were employed, namely, *Paramoecium* and *Nyctotherus cordiformis*. The former organisms were obtained from a culture grown in a solution of malted milk (1 gr. per 1,000 cc.), while the latter were obtained from the rectal contents of starved frogs *(Hyla aurea)*, which occasionally swarm with *Nyctotherus cordiformis*.

The following mitochondrial technique was employed during this investigation: All material was fixed in either a Flemming solution without acetic, or else in a Champy fluid, and afterwards washed out in running water for several hours following a 24 hours fixation.

These small organisms were then concentrated by means of centrifuging, brought up through the alcohols, cleared, and finally embedded *en masse* in paraffin, and afterwards sectioned 3–5μ in thickness. The sections were then stained by Heidenhain's iron-haematoxylin process, and occasionally counterstained in eosin. Examination of cut and stained sections with high magnifications through the mid-nuclear region of both these organisms show numerous small, rod-shaped, bacteria-like bodies, together with a lesser number of small spherical individuals, scattered irregularly throughout the cytoplasm; a denser accumulation of granular bodies around the outer surface of the nuclear

membrane, such as has already been described in a previous paper (1), may also be observed. When, however, surface sections of these protozoa are examined, it will be seen quite clearly that these rod-like bodies present a striking orientation in the surface cytoplasm of these cells.

That these bacteria-like bodies are mitochondria, and not symbiotic or parasitic organisms, is clearly shown by their subsequent reaction to certain stains and fixatives. Bacteria may be distinguished from rod-like mitochondria by exposing them for several hours in strong alcohol. Bacteria after such treatment stain up very sharply after staining with the Heidenhain process, while mitochondria, on the other hand, are rendered non-stainable by iron-haematoxylin after quite a short fixation.

In fact, any fixative containing large proportions of either acetic acid or alcohol render mitochondria non-stainable. It is also well known that the great majority of successful bacterial fixatives contain large proportions of alcohol.

All material used in this investigation was finally tested by the alcohol process; and, moreover, these cytoplasmic inclusions, present in both organisms, coloured up a bluish-green when stained *intra vitam* with a janus green B. solution (1 : 8,000), and the results obtained show clearly that these rod-shaped, bacterial-like bodies are true mitochondria, reacting in a manner similar to mitochondria present in other animal and plant cells.

OBSERVATIONS AND DISCUSSION.

When *Nyctotherus* and *Paramoecium* in longitudinal sections, after staining with Heidenhain's iron-haematoxylin process, are examined, it is observed that

---

EXPLANATION OF PLATE.

All figures (except figure 3) are drawn from material fixed in a Flemming solution without acetic acid, or else in a Champy fixative. All sections were then stained with Heidenhain's iron-haematoxylin, and occasionally counter-stained in eosin.

Figure 1. Oblique surface section of an adult Nyctotherus, showing orientation of mitochondria. Note how the rod-like mitochondria are arranged one behind the other, exhibiting a more or less transverse polarity to the axis of the cell. Observe also the irregularity of mitochondria in the deeper parts of the organism. Several mitochondria may be seen in the dumb-bell shaped condition.

Figure 2. Surface section of Paramoecium, demonstrating the arrangement of mitochondria in the surface cytoplasm. Note how the mitochondria occur one behind the other, presenting a longitudinal polarity to the infusorian.

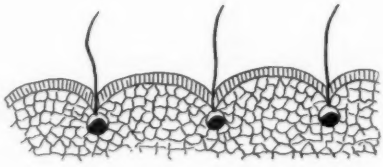
Figure 3. Transverse section through the outer region of an infusorian, demonstrating the position of the myoneme threads. Note the corrugations in the cytoplasm between each intervening myoneme thread. (After Butschli.)

Figure 4. Longitudinal section through the mid region of the nucleus of Nyctotherus, showing mitochondria scattered irregularly throughout the cytoplasm, together with large granular masses of mitochondria lying round the outer surface of the nuclear membrane. Micronucleus and apparently empty food vacuoles clearly visible.

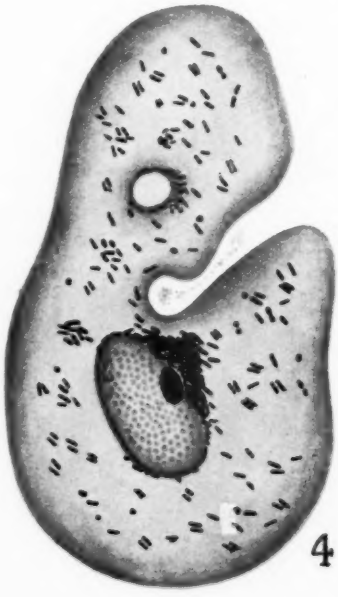
Figure 5. Longitudinal section of an adult Paramoecium cut through middle of cell, showing mitochondria aggregated around outer surface of meganucleus. Large numbers of mitochondria are also seen scattered throughout the protoplasm. Micronucleus and food vacuole containing mitochondria are clearly seen.

1

2



3



4

5

the cytoplasm of these organisms contains a large number of rod-shaped mitochondria, together with a smaller percentage of spheroidal bodies scattered throughout the protoplasm, the rounded individuals arising from the rod-shaped mitochondria by means of a transverse binary fission.

If, however, a section cut through the middle of the meganucleus be examined, it will be observed that the mitochondria are scattered in fairly large numbers, irregularly throughout the cytoplasm, while lying quite close to, and apparently in intimate contact with, the outer surface of the nucleus, may be observed a granular mass of mitochondria (see figure 4).

While examining the surface sections of these organisms I further observed that the rod-like mitochondria present in the surface cytoplasm exhibit a striking orientation; and at the same time a closer observation revealed the mitochondria in greater abundance in this region of the infusorian than in the centre. (See figure 1.)

A separate examination of *Nyctotherus* and *Paramoecium* demonstrates a marked difference in the arrangement of the orientation of the mitochondria in the surface cytoplasm of these two organisms. (See figures 1 and 2.)

If a surface section of *Nyctotherus* showing the orientation of mitochondria be observed under magnifications, it will be seen that the rod-like mitochondria are arranged one behind the other in longitudinal rows, each single mitochondrium exhibiting a polarity more or less transverse to the axis of the cell; while on the other hands an examination of the orientation of the mitochondria in *Paramoecium* shows that the rod-like mitochondria are arranged one behind the other also in longitudinal rows, but in this case the mitochondria present a longitudinal polarity to the axis of the infusorian.

A more detailed study of the surface sections of both these organisms reveals the presence of myoneme threads, which show up quite clearly after the cell has been treated with the ordinary mitochondrial fixatives. These myoneme threads run parallel to and beneath the rows of cilia, and according to Minchin (2) are a characteristic feature of the ciliate infusoria.

Owing to the presence of these myoneme threads, which, as has already been stated, run in longitudinal rows parallel to the axis of the cell, the surface cytoplasm is thrown into corrugations or folds. (See figure 3.)

Now in a previous paper (1) I have pointed out that the surface accumulation of mitochondria in cells may be interpreted in the light of their phosphatidal composition; and the same theory may be brought forward to explain how they come to lie round the inner surface of the cell membrane of the organisms at present under discussion. They would naturally accumulate in the corrugations, and the intervening myoneme threads would cause their segregation into distinct longitudinal rows.

It has been observed that in different infusorians the number and proximity of these myoneme threads varies. In *Paramoecium*, for example, they are so

closely adjacent to one another that the mitochondria are only able to lie end to end, thus giving rise to a longitudinal orientation. In *Nyctotherus*, on the contrary, the distance between each myoneme thread is greater, thereby allowing the rod-like mitochondria to lie side by side in a position transverse to the axis of the cell.

Thus it is strongly suggestive that the difference in the orientation in these two infusorians may be due to differences in the proximity of these myoneme threads to one another.

SUMMARY.

The orientation of mitochondria in the surface cytoplasm of infusorians may be correlated with their phosphatidal nature, together with the presence of myoneme threads.

This investigation was carried out during the tenure of a Government research scholarship in Zoology at the University of Melbourne.

REFERENCES.

1. Horning, E. S.: Aust. Journ. of Exper. Biol. and Med. Science, 4 (1927), p. 75.
2. Minchin, E. A.: Introduction to the Study of Protozoa (1922), p. 57.